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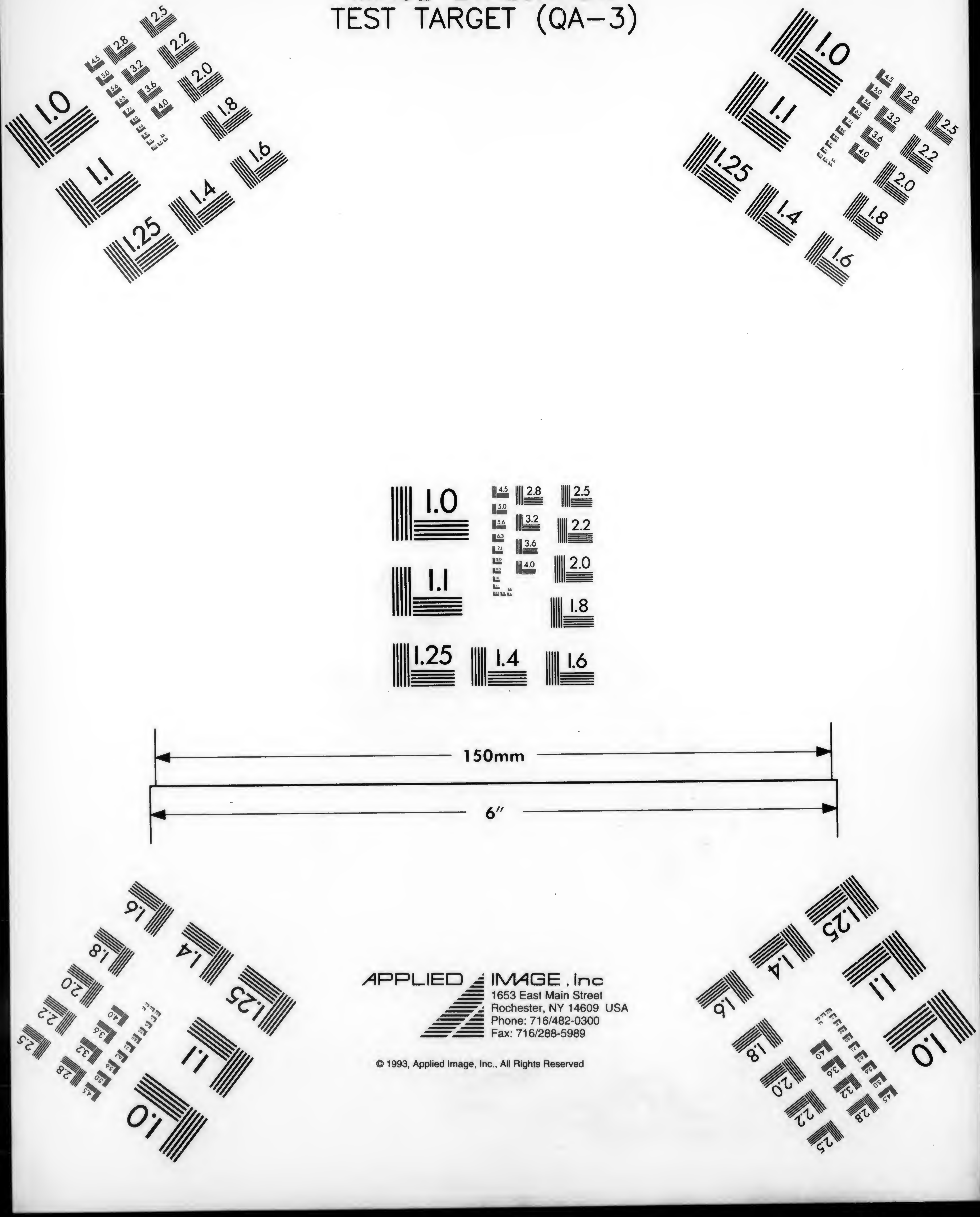

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THE PROGRESS OF THE DISTRIBUTION OF SALT IN HAM DURING THE CURING PROCESS

BY

R. C. MILLER AND P. T. ZIEGLER
(Contribution from Pennsylvania Agricultural Experiment Station)

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THE PROGRESS OF THE DISTRIBUTION OF SALT IN HAM DURING THE CURING PROCESS[1]

By R. C. MILLER, *assistant professor of agricultural and biological chemistry, Department of Agricultural and Biological Chemistry*, and P. T. ZIEGLER, *professor of animal husbandry, Department of Animal Husbandry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Practically all methods of curing pork involve the use of salt as a curing agent. Its main function is as a preservative to prevent or reduce the action of micro-organisms which might cause spoilage in the meat. Aside from this preserving action, the salt contributes greatly to the palatability of the meat. Oversalted pork is undesirable, although it is quite common. The amount of salt present should be sufficient to exert the required preserving action but should not be so excessive as to impair the palatability of the product. In addition the salt should be uniformly distributed throughout the meat to insure uniform quality and palatability.

The process of curing ham involves the application of the curing agent, usually a combination of salt, sugar, and either saltpeter (KNO_3) or potassium nitrite (KNO_2), to the meat by one of two methods, either by dry curing or brine curing. The meat is then allowed to remain in the curing medium for a period of time, which is dependent on the size of the cut and the kind of cure. Subsequently it is soaked in water to remove the excess salt from the outside, smoked, and finally aged or held as long as is desirable to improve its flavor or as is required for practical purposes.

The curing process has as its first objective the penetration of the salt into all parts of the meat rapidly enough to overcome or prevent the deleterious effect of micro-organisms. If the meat is dry-cured an allotted amount of the curing agent is rubbed into it, usually in two or three portions at intervals of several days. Usually the last portion is applied no later than 10 days after the beginning of the cure. In brine curing, which is probably more commonly used, the meat is allowed to remain in a brine or pickle for a period, the length of which should depend on the strength of the pickle used and the temperature at which the cure is conducted. Commercially it is a common practice to pump a strong salt solution into the ham previous to placing it in the pickle proper.

Other things being equal, the effectiveness of salt in preventing bacterial action should depend largely on the temperature at which the curing process is conducted. The optimum temperature is one which is high enough to permit the salt to penetrate as rapidly as possible, and yet low enough to inhibit excessive bacterial action. Temperatures from about 36° to 40° F. seem to fulfill this requirement and are commonly used. Lower temperatures are said to retard the cure,[2] and at higher temperatures spoilage of the meat is liable

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[1] Received for publication Sept. 16, 1935; issued March 1936.

[2] MOULTON, C. R. MEAT THROUGH THE MICROSCOPE; APPLICATIONS OF CHEMISTRY AND THE BIOLOGICAL SCIENCES TO SOME PROBLEMS OF THE MEAT-PACKING INDUSTRY. 528 pp., illus. Chicago, 1929. See p. 45.

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to occur before a sufficient concentration of salt is obtained to check bacterial action.

The authors have in progress a study of the factors involved in the curing of ham and bacon. The present paper reports their findings in experiments made to determine the degree of distribution of salt in hams cured by different methods, the salt distribution being related to the time allotted to the curing process proper and the time of any subsequent holding or aging.

EXPERIMENTAL PROCEDURE

Fifty-nine hams weighing from 14 to 19 pounds were used for curing. The plan followed was, whenever possible, to put down together, in the curing agent, both the right and left hams from the same hog, and to remove this pair of hams from cure at the same time, then to soak them in water to remove excess salt or curing agent from the outside. One ham was analyzed immediately before being smoked, and the other was smoked and held for a designated number of days, when it was analyzed also. The latter ham gave information as to the effect of holding the ham for an additional period and served as a check on the first.

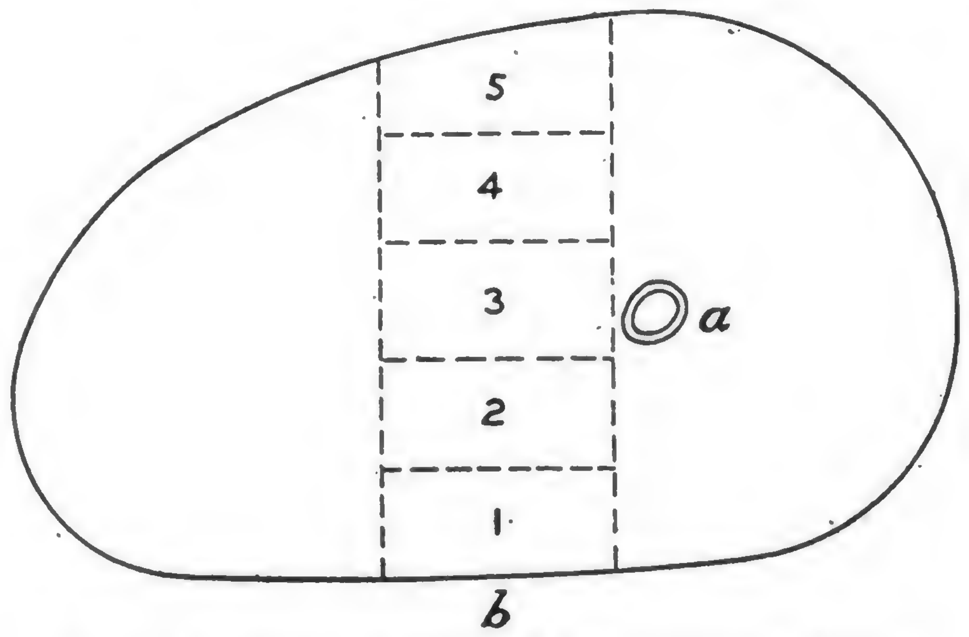

FIGURE 1.—Diagram of method of sampling the hams: *a*, Femur; *b*, skin side.

Each ham was sampled by taking a center cut across it next to the aitch bone.[3] This cut was about 1 inch thick, 3 inches wide, and as long as the thickness of the ham. It was divided into five equal horizontal sections which were separately ground in a food chopper. Each served as a separate sample on which moisture and chlorine were determined. Thus an analysis was obtained on samples representing the center of the ham as well as the two adjacent sections, and the two outside sections. This method of sampling is depicted in figure 1.

The amount of salt present in each section was calculated from the chlorine content as determined by the wet-digestion method as given by Davies.[4] A 5-gram sample of the ground meat was weighed into an Erlenmeyer flask, and to it were added an excess of one-tenth normal silver nitrate, 10 cc of saturated potassium permanganate solution, and 25 cc of concentrated nitric acid. This mixture was boiled vigorously until all solid particles of the meat were completely digested, the average time required being about 20 minutes. Perforated glass beads were used to prevent bumping during the digestion. The solution was then cooled by immersing the flask in cold water. When cool, the excess of silver nitrate was titrated with potassium thiocyanate in the presence of 2 cc of saturated ferric alum and 10 cc of acetone. By having a total volume of not more than 100 cc, an easily distinguishable brownish-red end point was obtained without filtering off the white precipitated silver chloride from the yellow solution resulting from the digestion. A blank determination on fresh pork showed it to be so low in chlorine as not to influence the results obtained in this study, in which relatively large quantities of salt were present.

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[3] MOULTON, C. R. See p. 112 of reference cited in footnote 2.

[4] DAVIES, W. L. THE DETERMINATION OF CHLORIDES IN DAIRY PRODUCTS AND BIOLOGICAL MATERIAL. Analyst 57: 79–85. 1932.

The percentage of salt in the dry matter of each of the five pieces that were subsampled from a ham was computed, and the figures obtained were used to calculate the mean salt content and the standard deviation of the center cut. It should be mentioned that the figures for subsample no. 1 were usually omitted in making the computations, inasmuch as this sample was ordinarily mostly fat and practically always contained less salt than the lean subsamples, presumably due to its lower water content. The coefficient of variability of the salt content was calculated according to the formula

$$cV = \frac{\sigma}{M} \times 100$$

in which cV represents the coefficient of variability.

The value thus obtained is a numerical expression which denotes the extent to which the salt contained in the ham has become distributed or equalized. It is a value which is practically independent of the absolute amount of salt present. Typical figures illustrating the application of this method of expressing the extent of salt equalization are given in table 1, the salt distribution for these two hams being quite different because of different methods of curing.

TABLE 1.—*Data obtained from the five subsamples made from center cuts of two hams cured by different methods and indicating the extent to which the salt was distributed throughout*[1]

Subsample no.—	Salt in ham no.—	
	1	2
	Percent	*Percent*
1 (fat side)	1.05	1.50
2	2.35	8.50
3 (middle piece)	2.89	8.97
4	9.87	8.46
5 (flesh side)	18.89	10.82
Mean salt content of entire center cut	7.01	7.65
Coefficient of variability	90.9	12.1

[1] All analyses are expressed as percentages of the dry matter.

As illustrated by these two hams, the coefficient of variability is high when the distribution of salt in a ham is not uniform, as in the case of ham no. 1, in which the salt is relatively more concentrated in subsamples 4 and 5. Conversely it will be low when the salt content of the several sections sampled is approximately the same, as in ham no. 2. Thus the coefficient of variability is a useful criterion in judging the extent of the distribution of salt and presents a means of comparing different hams in this respect.

It should be pointed out that the mean salt content of the five pieces subsampled from the center cut is not the true mean salt content of the whole ham. This is apparent when it is recognized that the outer portion of the ham (subsamples 5 and 1) represents, on the basis of weight, a different percentage of the whole ham than it represents in relation to the center cut from which it is taken. The same difference exists in a different proportion for the other subsamples of the center cut. As given in table 1 the mean salt content of the entire center cut is 7.01 percent for ham no. 1 while the salt content as determined for the whole ham after grinding it and subsampling was 5.36 percent. The method of center-cut sampling, therefore, is conventional but serves a useful purpose for comparative studies. In addition it serves as an accurate criterion when a consideration of salt penetration in relation to the thickness of the meat is desired, this aspect being equally as important as the relationships involving the weight of the meat.

In table 2 are given data concerning three different brands of standard, first-quality, packer's ham which are taken to be representative of this type of product. These hams were purchased in a local butcher shop and analyzed in the usual manner.

TABLE 2.—*Salt distribution in first-quality commercial hams of three different brands*

Part of ham or sample	Salt in ham no.—		
	1	2	3
	Percent	*Percent*	*Percent*
Middle sample (no. 3)	7.5	12.9	11.2
Outside sample (no. 5)	13.9	15.5	18.1
Mean salt content of entire center cut	11.6	15.8	14.0
Coefficient of variability for center cut	25.8	16.1	24.6

No information is at hand concerning the manner in which these hams were cured or how long they were out of the cure before being sampled. The analysis shows that they contained a considerable amount of salt and that, particularly in the case of hams 1 and 3, the salt content of the outside pieces was somewhat higher than that of the center sample. The coefficients of variability bear out this observation insofar as salt distribution is concerned. A value around 25 would indicate only a fair distribution; thus it is possible that at least two of these hams (nos. 1 and 3) were not held long after curing, or that some other feature of the cure was such as did not permit much equalization of the salt.

SALT DISTRIBUTION IN DRY-CURED HAMS

In table 3 are given data for hams cured under experimental conditions. Both groups were dry cured with salt and saltpeter. The hams having the same curing period were from the same hog.

The curing agent was applied at the rate of 1 ounce per pound to the skinback hams and 2 ounces per pound to the regular hams, a portion as large as could be immediately taken up by the meat being applied in one rubbing, the remainder being sprinkled on the meat. The curing was done in wooden boxes from which the liquor exuding from the hams was allowed to drain, and in a refrigerated room where the temperature was controlled to about 37° F. Pairs of hams were removed from the cure at the same time, one ham from each pair being immediately analyzed, and the other being held for an additional period, as indicated in the table, during which time it was smoked and then analyzed. The hams were held in a refrigerated room previous to smoking, and at room temperature after smoking.

TABLE 3.—*Salt distribution while curing and after holding in hams dry-cured for various periods with salt and saltpeter at 37° F.*

Ham no.[1]	Regular hams			Ham no.[1]	Skinback hams		
	Length of curing period	Length of holding period after cure	Coefficient of variability of salt		Length of curing period	Length of holding period after cure	Coefficient of variability of salt
	Days	*Days*			*Days*	*Days*	
7^3	20	0	123.8	3^4	28	0	92.8
1^3	35	0	100.9	10^4	35	0	98.5
5^3	42	0	81.9	2^4	42	0	76.7
10^3	20	30	52.8	5^4	28	32	14.0
2^3	35	30	25.8	1^4	35	32	36.0
3^3	42	30	34.4	6^4	42	32	20.0

[1] The superior figures are for laboratory records.

The results indicate that with both methods of curing the equalization of salt was progressing throughout the whole period of observation, which was from 20 to 74 days. However, the coefficients of variability are somewhat lower in five out of the six cases for the skinback hams, indicating a somewhat more rapid cure. The slower cure with the regular hams may be attributed to the heavier fat covering on the hams, this layer of fat retarding salt penetration.

It is apparent that the type of results presented in this paper show considerable variation because of the small number of hams that have been tested. Therefore, the results are of limited value in making close comparisons unless large numbers of observations are made, but are useful for the present purpose in indicating the general progress of salt penetration and equalization.

In table 4 are presented data for hams dry-cured with salt, brown sugar, and saltpeter, using 1 ounce per pound of meat, applied in three rubbings. One series of hams was kept during the cure in an outside brick building which was unheated, and with the temperature uncontrolled. The conditions simulated those that would be encountered in farm practice. The other series was cured under controlled conditions.

Salt alone or in combination with saltpeter is commonly used in farm practice for curing meats, but it is generally conceded that a product of better quality is obtained if the curing agent contains some sugar. Unpublished experimental work by the authors has shown that a mixture of 8 pounds of salt, 3 pounds of brown sugar, and 3 ounces of saltpeter is a satisfactory curing mixture.

Data for an odd ham are presented in the controlled series, its mate having been erroneously cut before the termination of the cure; also hams 2^A and R^2 were not from the same hog but were practically identical otherwise.

TABLE 4.—*Salt distribution, while curing and after holding, in hams dry-cured for various periods with salt, brown sugar, and saltpeter, under uncontrolled temperature conditions and at 37° C.*

Ham no.[1]	Cured with salt, brown sugar, and saltpeter, curing temperature uncontrolled. (40°–60° F.)			Ham no.	Cured with salt, brown sugar, and saltpeter at 37° F.		
	Length of curing period	Length of holding period after cure	Coefficient of variability of salt		Length of curing period	Length of holding period after cure	Coefficient of variability of salt
	Days	*Days*			*Days*	*Days*	
5^{5}	28	0	67.7	2^{A}	21	0	108.8
1^{5}	35	0	104.1	3^{2}	26	0	111.9
3^{5}	42	0	57.3	6	28	0	120.8
10^{5}	28	32	18.5	10^{2}	33	0	55.3
1^{5}	35	32	12.8	7	35	0	70.7
6^{5}	42	32	2.9	2^{2}	40	0	59.8
				5	42	0	48.7
				R^{2}	20	30	32.3
				6^{2}	26	30	12.7
				1	28	30	15.9
				2	35	30	12.2
				1^{2}	40	30	8.4
				3	42	30	11.0

[1] The superior numbers and letters indicate skinback hams. The other hams were regular.

In all the hams that were cured for 26 days or more and then held for 30 days, the distribution of salt, as judged by the coefficients of variability, was uniformly good. It appears that salt equalization was progressing at a rather uniform rate during both the curing and the holding periods and would be satisfactory after 50 to 60 days. Also there is some indication that the distribution of salt in these sugar-cured hams, as related to time, is somewhat better than in the case of hams cured with salt and saltpeter under controlled conditions. A comparison of hams that were cured for approximately the same periods and then held for a period after cure, shows that the coefficients of variability are lower in all cases for the sugar-cured hams.

SALT DISTRIBUTION IN BRINE-CURED HAMS

The results obtained with two series of brine-cured hams are given in table 5. The brine was made up of 8 pounds of salt, 3 pounds of brown sugar, and 3 ounces of saltpeter, dissolved in 4 gallons of water and used at the rate of 4 gallons per 100 pounds of meat. The series of hams, for which data are given in table 5, were in part cured under controlled temperature conditions at about 37° F., and in part under uncontrolled temperature conditions in the smokehouse previously described.

In the cases where comparisons are possible, it does not appear that there is much difference in salt equalization in the hams cured under either uncontrolled or controlled conditions.

The coefficients of variability of the salt of the hams cured in brine under controlled conditions are about the same for all the hams analyzed after 25 to 42 days in the brine. The figures for hams cured from 25 to 30 days appear to be lower than for those dry-cured for approximately the same period. It might be expected that such might be true since, in dry curing, all the salt which is ultimately distributed throughout the ham is rubbed into the surface within the first few days after the beginning of the cure. From then on the salt is penetrating further into the ham and is becoming distributed throughout it, the concentration of salt gradually being diminished in the outside portion. In brine curing, salt is being continually taken up by the meat from the brine until the cure in the brine is terminated. It would appear from the coefficients of variability, that in brine-cured hams the concentration of salt in the outside portion in relation to the concentration in the inside is never as great as it is in the early stages of dry curing. Because of the continued absorption of salt during the brine cure, equalization of the salt may not be as rapid as in dry curing during the cure proper. The data show that, in the case of brine curing, most of the equalization occurs during the holding period following the cure.

TABLE 5.—*Salt distribution in hams brine-cured for various periods, both at about 37° F. and under uncontrolled temperature conditions, while curing and after holding*

Ham no.[1]	Cured at about 37° F.			Ham no.	Cured under uncontrolled temperature conditions (40°–60° F.)		
	Length of curing period	Length of holding period after cure	Coefficient of variability of salt		Length of curing period	Length of holding period after cure	Coefficient of variability of salt
	Days	*Days*			*Days*	*Days*	
1^{B}	25	0	84.7	0^{A}	28	0	101.3
7	28	0	83.8	3^{A}	35	0	72.0
5^{E}	30	0	81.0	2^{C}	35	0	65.3
X^{E}	34	0	80.5	X^{C}	35	6	65.1
2	35	0	90.9	4^{A}	42	0	54.6
5	38	0	84.6	1^{C}	35	30	31.8
1	42	0	73.6	2^{A}	28	61	10.6
0^{B}	42	6	73.6	1^{A}	35	54	9.5
0	28	30	3.2	5^{A}	42	61	13.1
0^{E}	34	30	33.2				
2^{B}	35	32	17.1				
3^{B}	42	30	13.6				
3	35	64	17.8				
6	42	60	6.4				

[1] The superior letters are for laboratory records.

No mention has been made of the influence of smoking the meat on the salt penetration. It might be expected that exposure to the higher temperature of the smokehouse would hasten the equalization of salt, but as yet the present study has not particularly concerned this point and such evidence as has been obtained is inconclusive, although there is some indication that smoking slightly speeds up salt equalization.

With respect to the ultimate distribution of salt, the dry-curing and brine-curing processes are essentially the same. However, they must differ in fundamental details. Experiments are cited by Callow[5] in which small pieces of pork muscle were immersed in concentrated solutions of salt. At first, water diffused from the muscle to the solution, the rate of diffusion depending on the concentration of the salt solution. Finally, the water began to diffuse back from the strong solution of salt to the muscle. Apparently this phenomenon does not occur except in the presence of proteins, there being formed a salt-protein complex which has an osmotic pressure greater than that of the surrounding solution.[6] Since the salt-protein complex is hypertonic to the solution, water diffuses back into the protein-containing system.

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[5] CALLOW, E. H. THEORY OF CURING. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1932: 101–102. 1933.

Callow[7] has also shown that the uptake of salt from solutions of different strengths is continuous and is dependent on the strength of the brine used, more salt being taken up from the stronger solutions.

These findings agree with those of practice, the increase in weight of brine-cured hams due to uptake of water being commonly recognized. The results herein presented show, however, that under ordinary conditions the salt in a ham is not uniformly distributed for some length of time. It is doubtful, with a heterogeneous-phase composition with respect to salt and tissue constituents, whether equilibria with respect to salt and water uptake are reached in ordinary curing practice. Methods of curing, therefore, must be controlled procedures which produce the desired effect.

SUMMARY

A statistical method is presented, by means of which it is possible to express mathematically the extent of the distribution of salt in ham at various stages in the process of curing.

Results are given for 59 hams, variously cured.

The salt in hams cured by various methods did not become well distributed throughout the meat until approximately 60 days after the beginning of the various cures. Salt equalization begins in dry-cured hams as soon as the last portion of curing mixture is applied to the meat. In brine curing, salt equalization is accompanied by absorption of salt from the brine until the meat is removed from it, thereafter salt equalization alone takes place.

In all the curing methods used, the aging of hams for 30 days after their removal from the cure permitted further equalization of the salt and gave a product of more uniform salt content.

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[6] ADAIR, G. S. A THEORY OF PARTIAL OSMOTIC PRESSURES AND MEMBRANE EQUILIBRIA, WITH SPECIAL REFERENCE TO THE APPLICATION OF DALTON'S LAW TO HÆMOGLOBIN SOLUTIONS IN THE PRESENCE OF SALTS. Roy. Soc. [London], Proc. A 120: 573–603, illus. 1928.

[7] CALLOW, E. H. SECTION C.—PIG PRODUCTS. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1929: 67–73, illus. 1930. See p. 69.

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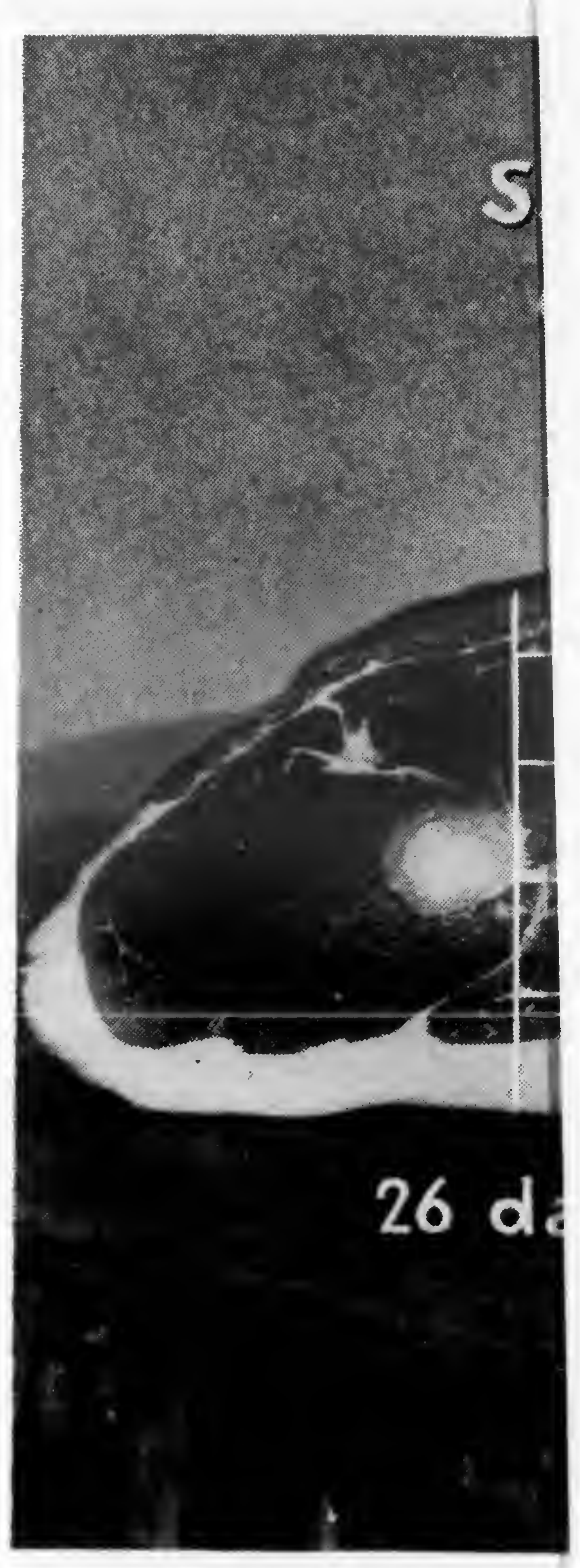

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content was also computed. From the mean and the standard deviation, the coefficient of variability of the salt content was computed, this value being a numerical expression which denotes the extent of variation in the salt content of the various sections analyzed.* Thus it expresses the extent of equalization or distribution of the salt between the five sub-samples.

This means of expressing the extent of the distribution of salt has been found to be very useful for comparative purposes. Thus if the salt contents of all five sub-samples of the center cut are approximately the same, a low co-efficient of variability of the order of from 5 to 20 will be obtained. If there is an excess of salt in any one or two of the sub-samples, as is often found, particularly in the outside portions of the ham, the coefficient of variability will be high, probably being between 50 and 150.

The analysis of the center cut samples of three first quality commercial hams are given in Table I, the percentage of salt given being that pres-

*The coefficient of variability is a measure of the dispersion of data as related to the average. It is expressed as the percentage of the mean which is represented by the standard deviation. Thus:

$$C.V. = \frac{\text{Std. Dev.}}{\text{mean}} \times 100$$

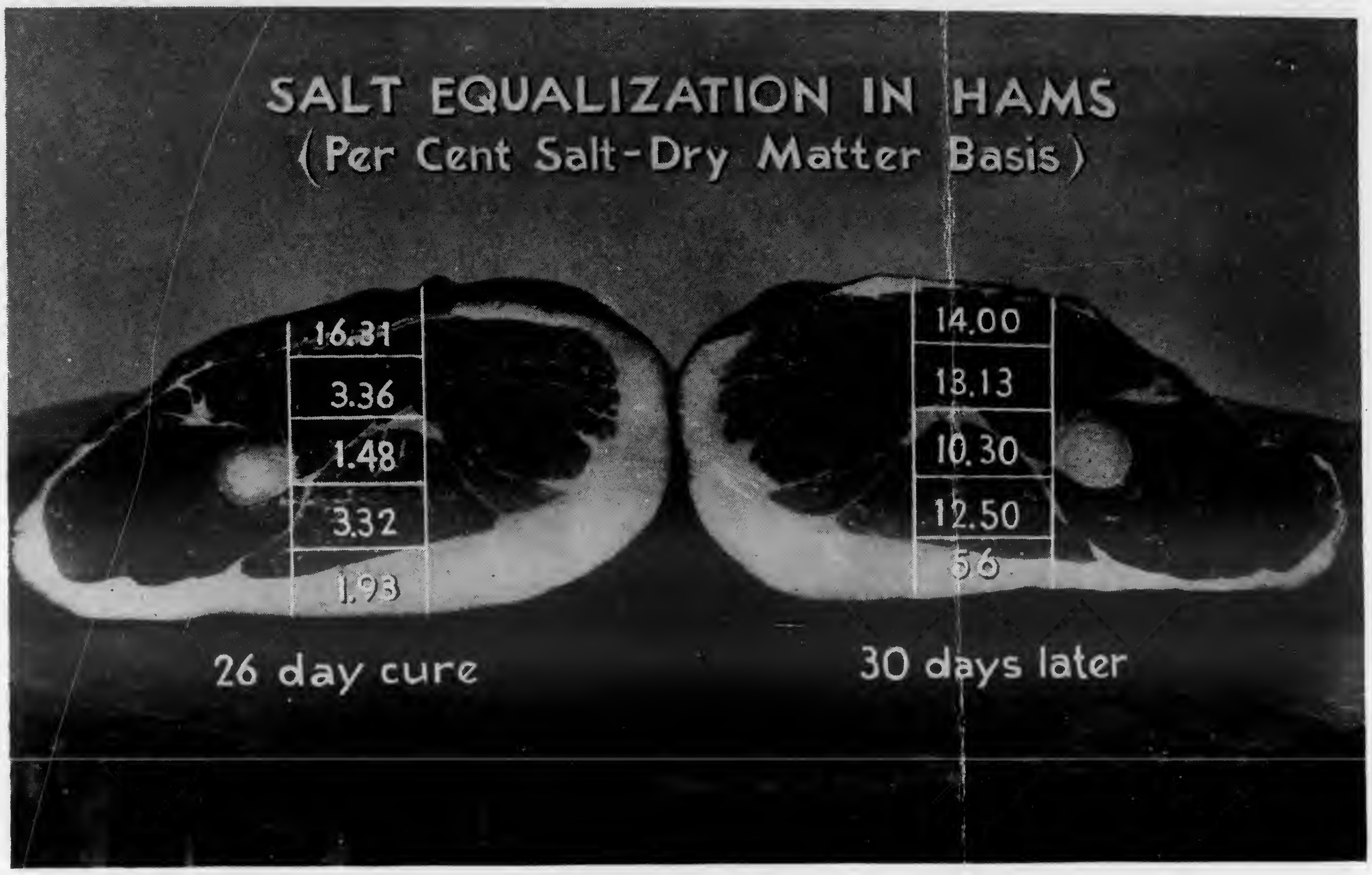
Table I: Per Cent of Salt Distr

Sample No. 1, skin side
Sample No. 2
Sample No. 3, center
Sample No. 4
Sample No. 5, outside
Mean salt content
Coefficient of variability of salt

Tabl

Method of cure	Number of days in cure
Dry-cured, packed in salt	14
Dry-cured, packed in salt	20
Brine-cured, 90° pickle	14
Brine-cured, 90° pickle	20
Brine-cured, 70° pickle	14
Brine-cured, 70° pickle	20
Brine-cured, 70° pickle	28
Brine-cured, 70° pickle	35
Brine-cured, 70° pickle	42

Table III: Dry-Cured



The rectangles shown on these hams by white lines indicate where the test pieces were cut.

How Aging Affects the Distribution of

Salt in Cured Hams

By R. C. MILLER and P. T. ZIEGLER
The Pennsylvania State College, State College, Pa.

THE time element is an important factor in securing a uniform distribution of curing materials used in cured hams. It is true that preservation of the meat by preventing or reducing the action of microorganisms is the principal object of the process of curing ham. However, in addition to this function, the curing agent contributes to the final quality of the meat as manifested by its color, flavor, tenderness, and saltiness. This is not to say that curing alone can produce a high quality product from inferior pork, but rather to emphasize that a poor quality can result from faulty curing methods applied to good quality pork. It goes without saying that a ham containing enough salt to preserve it well, may not be acceptable from the standpoint of palatability if the salt is not distributed uniformly throughout the piece.

The requirements with respect to salt in cured ham are: (1) that the amount present be sufficient to exert the required preserving action in all parts of the ham; (2) that the amount present should not be in large excess so that the ham would be said to be oversalted; (3) that the salt be uniformly distributed throughout the meat.

IN an investigation of the latter point, the idea in mind was that, with other things being equal, such as the curing temperature and the amount of curing agent that is applied or allotted to the meat, the completeness of the distribution of the salt is definitely related to the time elapsing after the beginning of the cure.

In carrying out some experiments concerning this point hams weighing from 14 to 19 lb. were cured. At some time during the cure each ham was sampled for salt analysis by taking a center cut across it next to the aitchbone, the cut being about one inch thick, three inches wide and as long as the ham was thick. This cut was divided into five equal horizontal sections which were separately analyzed for salt. Thus the salt content was determined on samples representing the center of the ham as well as the two adjacent sections, and the two outside sections, one from each side of the ham as shown in the photograph above.

The average salt content of the center cut was computed from the salt contents of the individual pieces. The standard deviation of the salt

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content was also computed. From the mean and the standard deviation, the coefficient of variability of the salt content was computed, this value being a numerical expression which denotes the extent of variation in the salt content of the various sections analyzed.* Thus it expresses the extent of equalization or distribution of the salt between the five sub-samples.

This means of expressing the extent of the distribution of salt has been found to be very useful for comparative purposes. Thus if the salt contents of all five sub-samples of the center cut are approximately the same, a low co-efficient of variability of the order of from 5 to 20 will be obtained. If there is an excess of salt in any one or two of the sub-samples, as is often found, particularly in the outside portions of the ham, the coefficient of variability will be high, probably being between 50 and 150.

The analysis of the center cut samples of three first quality commercial hams are given in Table I, the percentage of salt given being that present in the dry matter. It is seen that the lowest coefficient of variability is that for ham No. 3, where there is the least difference in salt content between the various sub-samples. It should be explained that sub-sample No. 1, being mostly fat, is not always used in the computation of the coefficient of variability. The salt in these three hams would be judged to be fairly well distributed throughout the meat, although it is most uniformly distributed in ham No. 3. Inasmuch as no information was available concerning the manner in which these hams were cured, it is not known what factors may have determined the distribution of salt in them.

In Table II are given some results obtained with hams cured by the authors. It is recognized that most of these hams were subjected to cure for very short periods and admittedly were not well cured, but they are presented here for purposes of illustration. None of these hams were smoked.

The results show how at the end of 14 days in cure the salt is relatively concentrated in the outer portion of the ham. Gradually some of this salt penetrates to the inner portion where its concentration is increased. A numerical picture of this phenomena is given by the coefficients of variability which decrease in value as the salt becomes equalized.

THESE facts are further illustrated by the results given in Tables III and IV. Pairs of hams from the same hog were put down in cure at the same time, one analyzed upon their removal from cure and the other held for 30 days before being analyzed, during which time it was smoked.

The results given in Tables III and IV show that if given time enough, the salt in a ham will become well equalized throughout the various portions. With hams cured from 26 to 42 days an additional aging of 30 days gave a product in which the salt was well distributed.

Further, removal of the ham from the cure proper, soaking it, and smoking do not stop the process of salt equalization, but rather it continues even through the process of smoking and subsequent aging. Smoking may possibly hasten salt equalization due to the exposure of the meat to a higher temperature. Experimental hams cured differently from those cited above had coefficients of variability of 5 per cent or less after combined curing and aging periods of from 120-130 days.

Apparently, from all of the results obtained so far, the method of curing has little influence on the salt equalization during the subsequent aging period. Dry salting and brine methods differ in fundamental details, however. In dry curing the salt is all applied to the meat within a short time after the starting of the cure, and thereafter the process is simply one of equalization and distribution of the salt throughout the meat. In brine curing, salt is being taken up from the brine continuously until the meat is removed from it, and not until this time does absorption of salt cease and salt equalization continue by itself. In either case additional aging after removal from the cure helps to produce a product uniform in salt content.

Table I: Per Cent of Salt Distribution in Commercial Hams

	Ham Number		
	1	2	3
Sample No. 1, skin side	8.7	2.9	6.6
Sample No. 2	11.1	11.4	16.5
Sample No. 3, center	11.2	7.5	12.9
Sample No. 4	15.4	13.7	18.1
Sample No. 5, outside	18.1	13.9	15.5
Mean salt content	12.9	9.8	13.9
Coefficient of variability of salt	24.6	25.8	16.1

Table II

Method of cure	Number of days in cure	Coefficient of variability of salt	Salt in center, sub-sample (per cent)	Salt in outside, sub-sample (per cent)
Dry-cured, packed in salt	14	117.5	0.6	2.5
Dry-cured, packed in salt	20	136.9	1.4	27.7
Brine-cured, 90° pickle	14	124.1	1.0	20.3
Brine-cured, 90° pickle	20	113.8	2.1	26.4
Brine-cured, 70° pickle	14	157.4	0.8	21.6
Brine-cured, 70° pickle	20	134.3	1.4	24.7
Brine-cured, 70° pickle	28	101.3	1.4	13.8
Brine-cured, 70° pickle	35	65.3	2.8	13.4
Brine-cured, 70° pickle	42	54.6	3.8	13.6

Table III: Dry-Cured Hams

(*Curing mixture = 8 lb. salt, 3 lb. brown sugar, 3 oz. saltpeter*)

Number of days in cure	Number of days held	Coefficient of variability of salt	Salt in center, sub-sample (per cent)	Salt in outside, sub-sample (per cent)
26	..	111.9	1.5	16.3
26	30	12.7	10.3	14.0
28	..	120.8	2.5	13.3
28	30	15.9	8.8	11.9
35	..	70.7	3.1	12.3
35	30	12.2	9.0	10.8
40	..	59.8	3.7	13.5
40	30	8.4	9.7	11.8
42	..	48.7	6.0	15.7
42	30	11.0	10.7	9.7

Table IV: Brine-Cured Hams

(*Curing mixture = 8 lb. salt, 3 lb. brown sugar, 3 oz. saltpeter in 4 gal. water, used for 100 lb. meat*).

Number of days in cure	Number of days held	Coefficient of variability of salt	Salt in center sub-sample (per cent)	Salt in outside sub-sample (per cent)
28	..	83.8	2.1	13.6
28	30	3.2	9.3	9.9
34	..	80.5	3.1	16.8
34	30	33.2	7.0	11.8
35	..	90.9	2.9	18.9
35	30	17.8	10.6	7.5
42	..	73.6	3.2	16.3
42	60	6.4	12.2	10.6

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*The coefficient of variability is a measure of the dispersion of data as related to the average. It is expressed as the percentage of the mean which is represented by the standard deviation. Thus:

$$\text{C.V.} = \frac{\text{Std. Dev.}}{\text{mean}} \times 100$$

ent in the dry matter. It is seen that the lowest coefficient of variability is that for ham No. 3, where there is the least difference in salt content between the various sub-samples. It should be explained that sub-sample No. 1, being mostly fat, is not always used in the computation of the coefficient of variability. The salt in these three hams would be judged to be fairly well distributed throughout the meat, although it is most uniformly distributed in ham No. 3. Inasmuch as no information was available concerning the manner in which these hams were cured, it is not known what factors may have determined the distribution of salt in them.

In Table II are given some results obtained with hams cured by the authors. It is recognized that most of these hams were subjected to cure for very short periods and admittedly were not well cured, but they are presented here for purposes of illustration. None of these hams were smoked.

The results show how at the end of 14 days in cure the salt is relatively concentrated in the outer portion of the ham. Gradually some of this salt penetrates to the inner portion where its concentration is increased. A numerical picture of this phenomena is given by the coefficients of variability which decrease in value as the salt becomes equalized.

THESE facts are further illustrated by the results given in Tables III and IV. Pairs of hams from the same hog were put down in cure at the same time, one analyzed upon their removal from cure and the other held for 30 days before being analyzed, during which time it was smoked.

The results given in Tables III and IV show that if given time enough, the salt in a ham will become well equalized throughout the various portions. With hams cured from 26 to 42 days an additional aging of 30 days gave a product in which the salt was well distributed.

Further, removal of the ham from the cure proper, soaking it, and smoking do not stop the process of salt equalization, but rather it continues even through the process of smoking and subsequent aging. Smoking may possibly hasten salt equalization due to the exposure of the meat to a higher temperature. Experimental hams cured differently from those cited above had coefficients of variability of 5 per cent or less after combined curing and aging periods of from 120-130 days.

Apparently, from all of the results obtained so far, the method of curing has little influence on the salt equalization during the subsequent aging period. Dry salting and brine methods differ in fundamental details, however. In dry curing the salt is all applied to the meat within a short time after the starting of the cure, and thereafter the process is simply one of equalization and distribution of the salt throughout the meat. In brine curing, salt is being taken up from the brine continuously until the meat is removed from it, and not until this time does absorption of salt cease and salt equalization continue by itself. In either case additional aging after removal from the cure helps to produce a product uniform in salt content.

bution in Commercial Hams

	Ham Number		
	1	2	3
.	8.7	2.9	6.6
.	11.1	11.4	16.5
.	11.2	7.5	12.9
.	15.4	13.7	18.1
.	18.1	13.9	15.5
.	12.9	9.8	13.9
.	24.6	25.8	16.1

II

Coefficient of variability of salt	Salt in center, sub-sample (per cent)	Salt in outside, sub-sample (per cent)
117.5	0.6	2.5
136.9	1.4	27.7
124.1	1.0	20.3
113.8	2.1	26.4
157.4	0.8	21.6
134.3	1.4	24.7
101.3	1.4	13.8
65.3	2.8	13.4
54.6	3.8	13.6

Hams

Table IV: Brine-Cured Hams

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THE EFFECT OF ELECTROLYTES PRESENT IN THE GROWTH MEDIA UPON THE ELECTROPHORETIC MOBILITY OF ESCHERICHIA COLI[1]

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I. INTRODUCTION

A number of articles have been published (Winslow, *et al.* 1923, 1926) in which the effects of electrolytes upon the electrophoretic mobility of bacteria have been reported. In these studies the organisms were grown, separated from the growth medium, suspended in the electrolyte solution under investigation, and the velocity of migration determined. The presence of low concentrations of electrolytes increased the mobility while higher concentrations decreased the mobility. Analogous results for non-living adsorbents are well known.

Would similar results be obtained if bacteria were grown in the presence of electrolytes or would the bacterial metabolism exercise some control over the mobility? This study attempts to determine this point and is of importance in the discussion of possible relationships between electrophoretic mobility and any bacterial property or activity.

II. EXPERIMENTAL PROCEDURE

A. The suspension. Escherichia coli was grown in a 2 per cent aqueous solution of Bacto-Peptone to which had been added the amount of salt necessary to give the desired concentration. The cultures were kept overnight at 37°C. and centrifuged for one hour. The supernatant liquid was poured off, the cells thoroughly mixed with distilled water, and again centrifuged. After the desired number of washings the bacteria were suspended in a small volume of distilled water, filtered through a small washed plug of cotton, and placed in a refrigerator. Before use each suspension was diluted with distilled water to the concentration most desirable for electrophoretic readings.

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[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, June 11, 1935, as Technical Paper 692.

[2] Taken from a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy at The Pennsylvania State College.

B. The determination of mobility. The mobility was determined with a Northrop-Kunitz micro-cataphoresis cell under a potential gradient of approximately 8.8 volts per centimeter. The microscope was fitted with a dark-field condenser and gave a magnification of 460 diameters.

The readings were made in the upper half of the cell at that depth (0.211) which gives actual electrophoretic velocities. For each sample the cell was filled twice, the microscope focused five times and 20 stopwatch readings taken at each focus. The average of these 100 readings was calculated to micra per second per volt per centimeter.

Control determinations were made with untreated bacteria at the beginning and at the end of each set of samples. Because of the excellent agreement between values obtained for the two controls, however, the second came to be considered unnecessary.

C. Washing of bacteria. A study was made of the effect upon the electrophoretic mobility of washing the organisms with water. This was done with bacteria grown in media containing no added electrolyte (blanks) and in media containing $CaCl_2$ and Na_2SO_4. Except in this study all cultures were washed once.

D. Growing time. To ascertain the precision with which the growing time would have to be controlled, an investigation of the mobility of organisms varying in age from 3 to 35 hours was made. All other cultures were grown approximately for 22 hours.

E. pH determinations. These were made colorimetrically using chlorophenol red.

III. EXPERIMENTAL RESULTS AND DISCUSSION

A. Washing studies. Organisms centrifuged from the growth medium and suspended in water were considered to have had "zero" washing. Other samples were washed 1, 2, 3, and 4 times. The results are given in table 1 and the averages in figure 1.

The values for the blanks are practically constant for 1, 2, 3, and 4 washings. For subsequent investigation, therefore, one washing was adopted as the standard procedure.

TABLE 1

Effect of washing upon electrophoretic mobility

	NUMBER OF WASHINGS				
	0	1	2	3	4
Blanks	1.66 1.82	2.23 2.16	2.23 2.22	2.18 2.25	2.34 2.22
Average	1.74	2.20	2.22	2.21	2.28
$CaCl_2$ 0.01 molar	0.94 0.96	1.86 1.60	2.05 1.81	2.27 2.05	2.24 2.20
Average	0.95	1.73	1.93	2.16	2.22
Na_2SO_4 0.5 molar	2.42 2.38	2.75 2.61	2.44 2.48	2.32 2.36	2.29 2.32
Average	2.40	2.68	2.46	2.34	2.31

Each determined value represents the average of 100 readings expressed in μ/second per volt/centimeter.

It appears from figure 1 that in the growth media the mobilities of the bacteria were less than those observed for "zero" washing. It also appears that one washing removes the electrolytes in the case of the blank but not where $CaCl_2$ or Na_2SO_4 were used. If the calcium and the sulphate ions were preferentially adsorbed, their gradual removal by washing would explain the difference in behavior noted for $CaCl_2$ and Na_2SO_4.

The fact that the mobilities eventually obtained, through continued washing of the treated and untreated bacteria, were practically identical indicates that the salts had produced no irreversible change in the nature of the bacterial surface.

B. Growing time. Possible changes in electrophoretic mobility due to differences in the growing time were sought with media containing no added electrolyte and with media 0.1 molar with respect to Na_2HPO_4. The results are given in table 2.

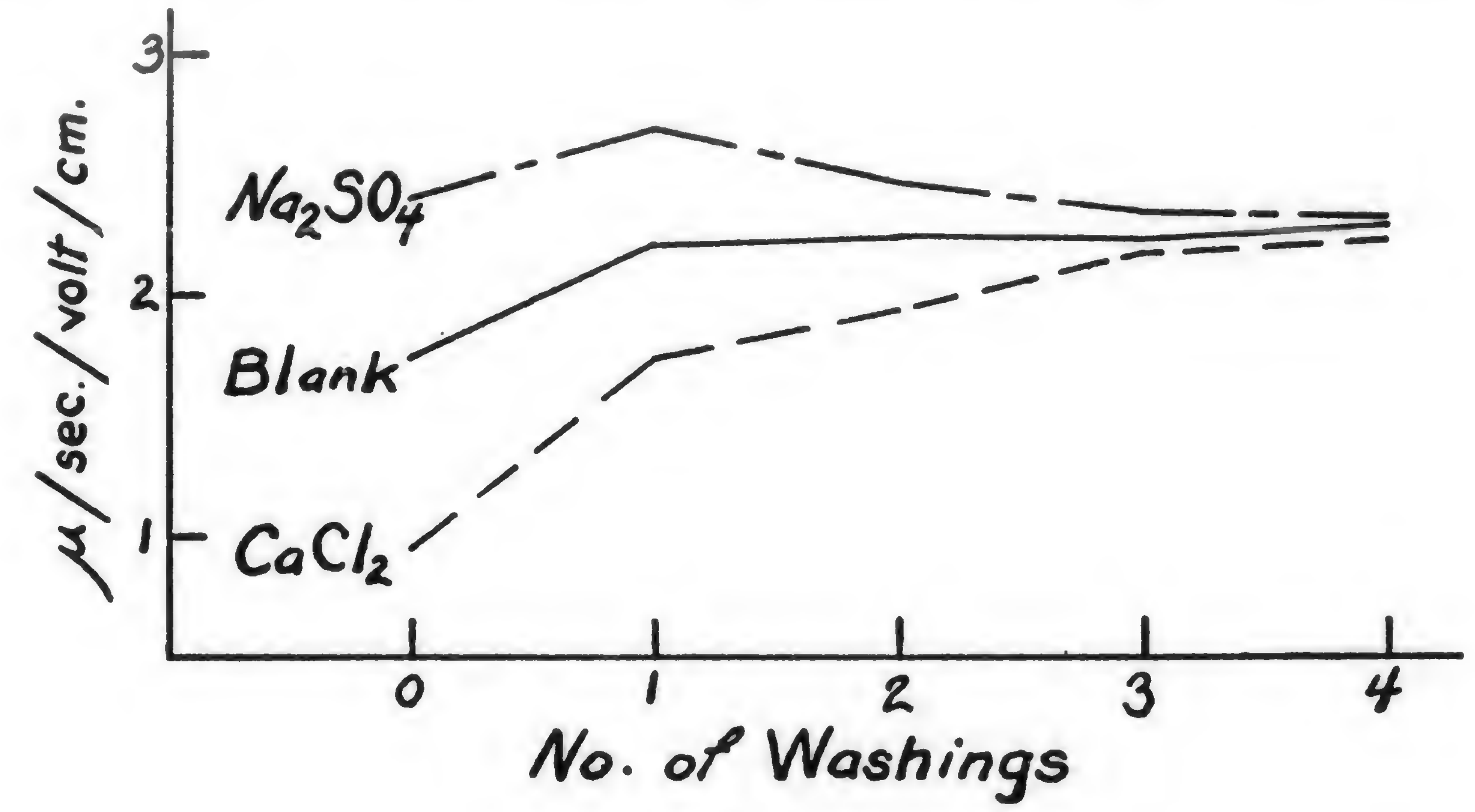

FIG. 1

TABLE 2

Relation of growing time to electrophoretic mobility

	3 HOURS	6 HOURS	10 HOURS	15 HOURS	21 HOURS	28 HOURS	35 HOURS
Blanks	2.29	2.22	2.22	2.26	2.27	2.26	
	2.20	2.22	2.25	2.26	2.24		2.26
Average	2.25	2.22	2.23	2.26	2.26	2.26	2.26
Na_2HPO_4 0.1 molar	1.73	1.61		1.73	1.87	1.90	
	1.70	1.69	1.85	1.84	1.89	1.96	
Average	1.72	1.65	1.85	1.78	1.88	1.93	

Each determined value represents the average of 100 readings expressed in μ/second per volt/centimeter.

The results for blanks show no change with growing time. Although the Na_2HPO_4-containing media produced a slight upward trend in migration velocity, the change is hardly significant when compared with the data reported by Shibley (1924) for a Type 1 *pneumococcus* and for *Salmonella paratyphi* B. He reported an increase in mobility to a maximum at about 10 hours followed by a decrease to a constant value after about 15 hours. Shibley's findings are apparently not of general applicability. Kahn and Schwarzkopf (1931) working with the tubercle bacillus found that younger cultures have a higher mobility than older cultures. The ages of their cultures varied, however, from four days to five weeks so that the results are not strictly comparable.

The increased mobility obtained might have been due to a decrease in the active concentration of Na_2HPO_4 brought about by bacterial metabolism rather than to any direct effect of the age of the culture.

C. pH values. It has not been possible to correlate the pH values secured with any changes in electrophoretic mobility. The pH values varied, as would be expected, with the multiplication of the bacteria; old cultures showed greater changes from the original than young cultures, and luxuriant growth produced greater changes than meager growth. After the growth of the bacteria the values were all on the alkaline side of neutrality with the maximum at pH 8.4. The data of Winslow and Upton (1926) show practically constant migration velocities for coli from pH 6.0 to pH 8.5 and the values obtained all fall within this pH range. Accordingly it is concluded that changes in migration velocity were not produced by changes in pH.

D. Salt studies. 1. Blanks. The results obtained for the blanks (read before the treated samples) show as an average of 2,740 stopwatch readings a migration velocity of 2.29 μ/seconds for unit potential gradient. The second blank (read after the treated samples) gave the same value for the average of 1,240 individual readings. Of the 62 blanks determined only one showed a greater deviation than ±5 per cent from the average value of 2.29 μ/seconds per volt/centimeter. This one value was 6.6 per cent too low. The conclusions are therefore drawn that the reading technic is adequate to give reproducible results, that differences in the data are not due to the reading

TABLE 3

Effect of salts on electrophoretic mobility

SALT	CONCENTRATION								CHANGE FROM BLANK AVERAGE 2.29
	0.00001M	0.0001M	0.001M	0.01M	0.05M	0.1M	0.5M	1.0M	
Na_2HPO_4		2.11 2.14 2.35 2.34	2.37 2.23 2.36 2.33	2.22 2.15 2.36 2.32	1.94 1.85 1.90 1.88	1.76 1.52 1.76 1.80	1.33 1.33		
Average...		2.24	2.32	2.26	1.89	1.71	1.33		Decrease
$CaCl_2$	2.23 2.25	2.17 2.13 2.15	2.14 2.13 2.09	1.83 1.82 1.79	1.76 1.52 1.34 1.33 1.75 1.74	1.76 1.52 1.32 1.65 1.71 1.76			
Average...	2.24	2.15	2.12	1.81	1.57	1.62			Decrease
NaCl		2.24 2.25	2.03 2.27 2.37	2.22 2.27	2.18 2.46 2.64 2.62	2.24 2.53 2.70 2.59	2.49 2.54 2.58 2.59	2.56 3.19 2.75	
Average...		2.25	2.22	2.25	2.48	2.51	2.55	2.83	Increase
Na_2SO_4		2.31 2.31	2.21 2.23	2.25 2.37	2.47 2.42	2.67 2.55 2.80 2.52	2.67 2.69 2.69 2.58	3.00 2.79	
Average...		2.31	2.22	2.31	2.45	2.63	2.66	2.90	Increase
NaI			2.29 2.27	2.24 2.23 2.24	2.17 2.21 2.26	2.13 2.14 2.28	2.15* 2.23*		
Average...			2.28	2.24	2.21	2.18	2.19*		Slight decrease

* 0.25M.

Each determined value represents the average of 100 readings expressed in μ/second per volt/centimeter.

technic, and that the organism can be grown over a long period of time without change in zeta potential.

2. Effect of salts. The results are given in table 3 and figure 2. The lowest values obtained (0.5 M Na_2HPO_4) represent a decrease in migration velocity of 41.9 per cent; the highest value (1.0 M Na_2SO_4), an increase of 26.5 per cent.

The results for the individual samples receiving the same treatment show in most cases a fairly good agreement one with another, but in a few instances, such as the higher concentrations of $CaCl_2$, considerable differences are evident. Attempts were made to correlate the different values with the age of the culture, the age of the suspension, the age of the distilled water used, and the pH of the growth medium, but no relationship could be established.

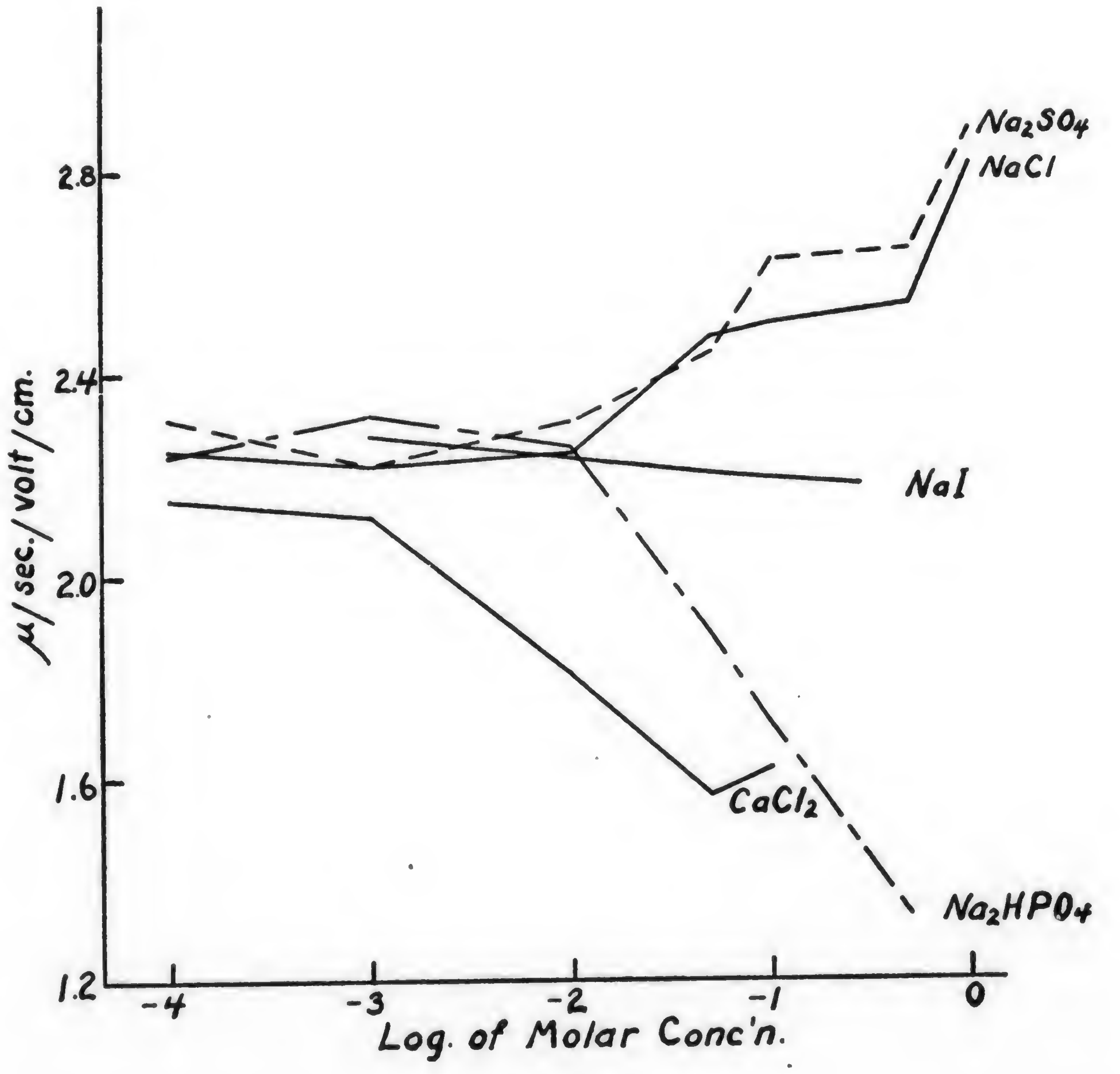

FIG. 2

No significant change in mobility has been accomplished by the addition of NaI to the growth medium. It is difficult to believe that this salt had no effect upon the mobility of the colon bacilli, but what did happen is not clear.

E. General discussion. Electrophoretic potential varies directly with the thickness of the electrical double layer and with the density of the charge on the particle. Abramson and Müller (1933) indicate that in low concentrations of electrolytes the charge per unit area is the more important factor in determining electrokinetic behavior, while in higher concentrations the thickness of the double layer is the more important factor. The explanation of the results obtained in the washing experiments seems to depend on both of these factors. In the various growth media the thickness of the double layer was very probably considerably decreased and the immediate result of replacing the growth medium by water (zero washing) should be an increase in the thickness of the double layer with a resultant increase in mobility. In subsequent washings the density of the charge on the particles seems to be the factor largely responsible for further changes in mobility.

From the increased mobility encountered with NaCl and Na_2SO_4 it appears that the high concentrations of these two salts in the growth media produce an increased charge density. This high charge density together with the increased thickness of the double layer, brought about by washing, would produce the high mobilities recorded.

The decreased mobility produced with such dissimilar salts as Na_2HPO_4 and $CaCl_2$ would seemingly be due to a decreased charge density. With the $CaCl_2$ a decrease in the negativity of the charge on the bacterial surface undoubtedly occurred, but whether the Na_2HPO_4 acted in a similar manner is not evident even though similar results were noted.

To explain adequately the differences found among the various salts used would require a knowledge of the relative adsorbability of the ions involved and also the active concentrations of these ions in the suspensions studied. In view of these considerations it is not surprising that the data presented do not follow the curves mentioned in the introduction.

The results obtained in this investigation raise a question as to the validity of many of the conclusions arrived at in bacterial electrophoresis. It has been a common practice to suspend washed bacteria in water and to determine the migration velocity of the organisms. From such data conclusions are drawn and classifications are made in attempts to correlate virulence, age of culture, strain of organism, etc., with electrophoretic mobility. The data accumulated here indicate the improbability of any direct relationship existing between the mobility of bacteria suspended in water and the mobility of these same bacteria in their normal environment.

IV. SUMMARY

A study was made to ascertain the effects upon the electrophoretic mobility of *Escherichia coli* of the presence of electrolytes in the growth media. The following conclusions seem justified.

1. The washing with water of colon bacilli grown in peptone broth and in peptone broth containing $CaCl_2$ increases the electrophoretic migration velocity to a constant value.
2. The washing with water of colon bacilli grown in peptone broth containing Na_2SO_4 first increases and then decreases the migration velocity to the same constant value mentioned in (1).
3. Contrary to results reported for other bacteria, the electrophoretic mobility of colon bacilli is not greatly influenced by the age of the culture up to 28 hours.
4. The technic used gives, for colon bacilli cultured over a considerable period of time in peptone solution, average values reproducible within ±5 per cent.
5. The pH changes which took place did not change the electrophoretic mobility.
6. Under the conditions of this experiment, the electrophoretic mobility can be increased or decreased by the presence of certain salts in the growth medium.
7. The well known mobility-concentration curves determined with salts present in the suspension media were not obtained in this study.

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A NEW ELECTROPHORESIS CELL FOR MICROSCOPIC OBSERVATIONS[1,2]

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PART I. DESCRIPTION, THEORY, AND THEORETICAL ADVANTAGES OF THE NEW CELL

Recent electrophoretic studies have involved the use of closed type cells, of either rectangular or circular cross section. The theory and use of such cells have recently been reviewed by Abramson (1). In both of these types the liquid returns along the axis of the cells, the velocity of the liquid being a parabolic function of the depth. The actual electrophoretic velocity can be obtained from observations at certain depths. However, at these depths the velocity gradient is large; the observed velocity changes rapidly with depth, and inaccuracy results.

The new cell described in this paper consists of two tubes in parallel between the electrodes, the dimensions of the tubes having a relation such that return flow takes place only through the tube of larger radius; there is no movement of the liquid along the axis of the tube of smaller radius, hence the velocities observed at the one-half depth in this tube are the actual electrophoretic velocities. At this depth the velocity gradient is zero, so that a slight inaccuracy in determining the depth of observation produces no appreciable change in the observed velocity of the particles.

DESCRIPTION OF APPARATUS

Diagrams of the apparatus are shown in figure 1. The essential parts of the apparatus are two fused quartz tubes, T_1 and T_2, used in parallel, two end tubes having stopcocks for filling the apparatus, and electrodes.

The dimensions of these two quartz tubes should have the relation

$$L_2/L_1 = A^2(A^2 - 2)$$

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[1] The data in this paper are taken from a thesis submitted by Margaret E. Smith to the Faculty of the Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1935.

[2] Authorized on July 27, 1935 for publication as Paper No. 698 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

where $A = R_2/R_1$, and L_2, L_1, R_2, and R_1 are the lengths and radii of tubes T_2 and T_1, respectively. The development of this equation is given in the section concerned with the theory of the cell.[3]

The procedure followed to obtain tubes of the correct dimensions was as follows: Tube 1 was ordered according to certain specifications,[4] viz., a capillary tube of good optical quality fused quartz, outer diameter approximately 3 mm., bore 0.5 – 0.7 mm., three sides of the tube to be ground off giving two parallel planes and one at right angles to these, thus allowing for observation, and for illumination either from the side or from underneath as desired. A diagram of the cross section of the tube is also shown in figure 1. The dimensions permissible for the cross section of this tube are limited by the working distance of the optical equipment to be used.

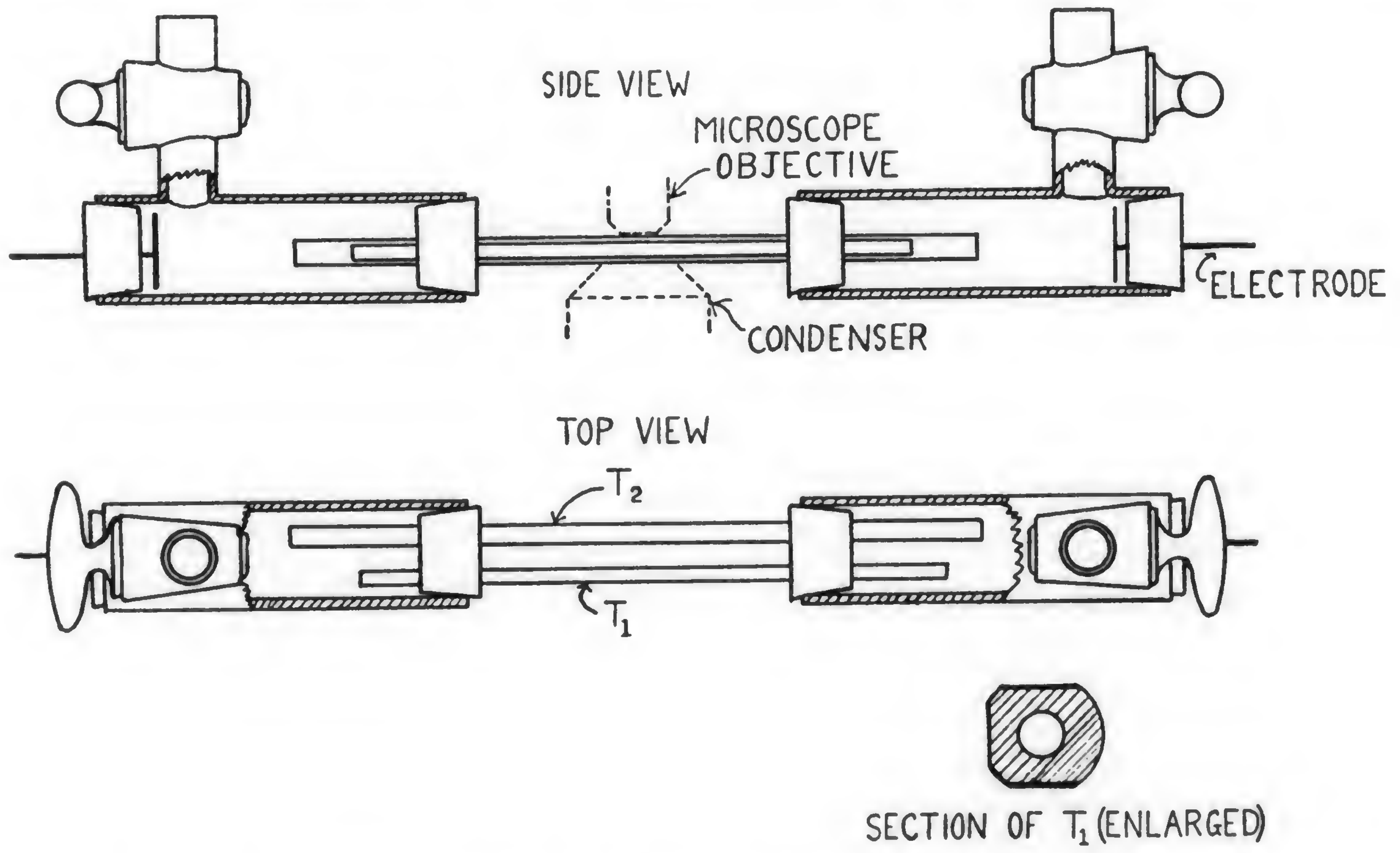

FIG. 1. Diagrams of the new double-tube cell for determination of electrophoretic mobilities

The bore of tube 1 was carefully determined by calculation from the length and weight of a column of mercury placed in the tube. Then a length of tubing was ordered from the stock supply of fused quartz tubing, with the specification that its bore should be uniform, and within the range of 1.5 to 1.6 times that of tube 1. The bore of this tube was determined in the same manner as described for tube 1, and the desired length cut off for tube 2.

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[3] The authors are indebted to Dr. Turner L. Smith of the Carnegie Institute of Technology, Pittsburgh, Pennsylvania, for the suggestion of the new cell and development of the theory concerning it.

[4] The quartz tubes were obtained from the Thermal Syndicate, Ltd., Brooklyn, New York.

Thus, if T_1 has a length of 10 cm., a radius of 0.3 mm., and T_2 has a radius of 0.48 mm., the length of T_2 is given by

$$L_2 = 10 \times 1.6^2(1.6^2 - 2)$$

or $L_2 = 14.34$ cm.

It is essential that tubes 1 and 2 be of the same material, since development of the theory of the cell assumes like surface conditions in the two tubes. Quartz was selected for these tubes, since (a) an optically good plane surface can be obtained through grinding and polishing of the quartz, and (b) the indices of refraction of quartz and water are more alike than those of glass and water, hence there should be less distortion of the images.

The quartz tubes were then inserted into two-hole rubber stoppers, making sure that the two tubes were far enough apart so that the larger tube would not interfere with the use of the objective and condenser on the microscope, and sealed into the stoppers with deKhotinsky cement.

The end tubes were of Pyrex glass tubing, with Pyrex glass stopcocks added for filling. The exact dimensions used are not important; in this case the length was approximately 12 cm. and the bore 1.8 cm.

The electrodes used were circular platinum disks, with wires attached leading through the rubber stoppers for connecting to the source of potential. In case solutions are used in which polarization occurs readily, nonpolarizable electrodes should be used.

OPERATION OF CELL

Operation of the cell involves the same technic as in the operation of other cells for microscopic observation of electrophoretic movement, except that the microscope is focused on the one-half depth in the tube of smaller radius. Hence the procedure is discussed here only in brief.

An apparatus resting on three leveling screws was built for holding the cell; this gave a simple means of adjustment for getting the plane of the cell parallel to the surface of the condenser. After the cell is filled with suspension and placed in position the electrodes are connected to the source of potential. A double-throw switch allows for frequent reversal of the current, thus preventing polarization. A milliammeter may be connected in series with the cell if desired.

The mobility of the particles (velocity per unit potential gradient) can be determined if the potential gradient is known. Since the radii of tubes T_1 and T_2 are much less than the radii of the end tubes, for most work the fall of potential through the end tubes can be neglected. Then the potential gradient through the tube where observation is made is given by E/L_1, where E is the total fall in potential between the electrodes, measured with a voltmeter in parallel with the cell, and L_1 is the length of T_1.

If a greater degree of accuracy is warranted, the potential gradient can be calculated more exactly from the dimensions of the apparatus. The use of this method in reference to electrophoretic cells is discussed by Northrop (3). Measurement of the current allows for another method of calculating the potential gradient; this method is discussed by Abramson (1).

For cleaning the apparatus, the rubber stoppers are removed from the end tubes. The two quartz tubes are left sealed in the two-hole stoppers. These tubes can be filled with water, alcohol, ether, or cleaning solution as desired with the aid of a hypodermic syringe; also, if desired, a small wire (insulated, thus preventing scratching of the surface) can be passed through the tubes. The end tubes and the electrodes are also cleaned. Thorough rinsing of the apparatus is very important, since small traces of electrolytes markedly affect electrophoretic potentials.

THEORY OF THE CELL

1. General theory of electrosmotic flow in tubes

The theory of electrophoresis cells assumes that the liquid is electrically neutral except for a charged layer lying very close to the wall of the cell. The electric field which is applied causes motion in this layer; this is defined as electrosmosis. Owing to the viscosity of the fluid, the motion of this layer induces a motion of the entire fluid in the cell. This motion is assumed to be both steady and nonturbulent.

The theory of tubular electrophoresis cells may be derived in the following manner. Consider the portion of the liquid inside a cylinder coaxial with the tube but of smaller radius. Electrosmotic movement of the liquid results in a difference in pressure at the ends of the tubes; the resultant force acting on the ends of the cylindrical portion of the fluid is just balanced by the shearing stress on the cylindrical surface, caused by the viscosity and thus proportional to the rate of change of velocity with the radius. This balance is expressed by the equation

$$2\pi r L \eta \frac{\mathrm{d}v_W}{\mathrm{d}r} = \pi r^2 P$$

which gives on integration

$$v_W = \frac{P}{4\eta L} r^2 + C \qquad (1)$$

in which v_W denotes the velocity of the liquid, P the difference in hydrostatic pressure at the ends of the tube of length L, η the coefficient of viscosity of the liquid, and r the radius of the tube of flow being considered. This equation is the fundamental equation for flow of liquids in tubes; its development is found in treatises concerned with hydrodynamics of liquids (see Lamb (2)).

In the case being considered, the constant C can be evaluated from the condition that the velocity of the liquid at the wall of the tube is the electrosmotic velocity; that is, when $r = R$, $v_W = V_eE/L$, where R denotes the radius of the tube, V_e the electrosmotic mobility of the liquid, E the applied electromotive force, and L, as above, is the length of the tube. Hence equation 1 becomes

$$v_W = \frac{P}{4\eta L}(r^2 - R^2) + \frac{V_eE}{L} \qquad (2)$$

The total volume, W, of liquid transported per unit time across any section is given by

$$W = \int_0^R v_W \cdot 2\pi r \, \mathrm{d}r$$

Substituting in this the value of v_W given in equation 2, and integrating, we obtain

$$W = \pi R^2 \left(\frac{V_eE}{L} - \frac{PR^2}{8\eta L} \right) \qquad (3)$$

Equations 2 and 3 are the general equations for the velocity of electrosmotic flow of liquids and the volume of liquid transported by electrosmosis in cylindrical tubes.

2. Theory of electrophoresis in a single closed tube

The observed velocity, $V_{\text{obsd.}}$, of a particle at any depth in the tube will be the sum of the actual electrophoretic velocity, U, and the velocity of the liquid, i.e.,

$$V_{\text{obsd.}} = U + v_W \qquad (4)$$

In a single closed tube, the total volume of liquid transported in one direction is zero. When $W = 0$, from equations 2 and 3 we obtain the equation for the velocity of the liquid in a closed tube

$$v_W = \frac{P}{4\eta L}\left(r^2 - \frac{R^2}{2} \right) \qquad (5)$$

The depths at which there is no movement of the liquid are found by setting $v_W = 0$ in equation 5; this gives

$$r = \pm \frac{R}{2}\sqrt{2}$$

Focusing at these depths, the observed velocity is the actual electrophoretic velocity of the particles.

Actual electrophoretic velocities can also be obtained in another manner, thus,

$$\int_0^R V_{\text{obsd.}} \cdot 2\pi r \, dr = \int_0^R U \cdot 2\pi r \, dr + \int_0^R v_W \cdot 2\pi r \, dr \tag{6}$$

In a closed tube the total volume of liquid transported, represented by the last term in the preceding equation, is zero; hence equation 6 becomes

$$U = \frac{2}{R^2} \int_0^R V_{\text{obsd.}} \cdot r \, dr \tag{7}$$

Therefore, by taking readings at various depths in the cell, an approximation of the actual electrophoretic velocity can be obtained.

By substituting $r = 0$ and $r = R$ in turn in equation 5, it appears that the velocity of the liquid at the axis of the tube is equal in magnitude but opposite in direction to the velocity of the liquid at the wall of the tube, i.e., to the electrosmotic velocity, V_eE/L. Hence by use of equation 4 we obtain

$$V_eE/L = U - V'_{\text{obsd.}} \tag{8}$$

where $V'_{\text{obsd.}}$ is the observed particle velocity in the single tube cell at depth $r = 0$. Thus the electrosmotic velocity can be calculated if the observed particle velocity at the one-half depth and the electrophoretic velocity are known.

3. Theory of cell consisting of two tubes in parallel

Consider two tubes of different radii in parallel between two electrodes. Let T_1 denote the tube of smaller radius, and T_2 that of larger radius. The general equations 2 and 3 for the velocity and volume of liquid transported apply; subscripts will be used to refer to the dimensions of these two tubes, as L_1 for the length of T_1, R_1 its radius, etc. The actual electrosmotic mobility, V_e, will be the same in both tubes, since the tubes are of the same material; also, the applied E.M.F. and the difference in pressure, denoted by E and P, respectively, will be the same in both tubes.

The total volume of liquid transported in one direction in the two tubes is zero; hence from equation 3 we obtain

$$W = \frac{\pi R_1^2}{L_1}\left(V_eE - \frac{PR_1^2}{8\eta}\right) + \frac{\pi R_2^2}{L_2}\left(V_eE - \frac{PR_2^2}{8\eta}\right) = 0 \tag{9}$$

We wish to determine conditions such that there will be no movement of liquid along the axis of the smaller tube. The velocity at this depth can be obtained by setting $r = 0$ in equation 2. This gives

$$v_W \text{ along axis of } T_1 = -\frac{PR_1^2}{4\eta L_1} + \frac{V_eE}{L_1} \tag{10}$$

When this velocity = 0,

$$V_eE = \frac{PR_1^2}{4\eta} \tag{11}$$

This value substituted in equation 9 gives on simplification

$$R_1^4 L_2 + 2R_2^2 R_1^2 L_1 - R_2^4 L_1 = 0$$

Solving this for L_2,

$$L_2 = \frac{L_1(R_2^4 - 2R_1^2 R_2^2)}{R_1^4} \tag{12}$$

Let the ratio of the radii, R_2/R_1, be denoted by A. Then equation 12 can be written in the form,

$$L_2/L_1 = A^2(A^2 - 2) \tag{13}$$

If tubes are used whose dimensions satisfy the relations given in equation 13, there will be no movement of liquid along the axis of the smaller tube; velocities observed at this depth will be the actual electrophoretic velocities of the particles.

THEORETICAL ADVANTAGES OF THE NEW CELL

According to the development of the theory as given, velocity of liquid in tubes due to electrosmosis is a parabolic function of the depth; this is evident from the general equation 2, which is of the type $v_W = Ar^2 + B$, the constants A and B being dependent on conditions in the tubes used.

Equation 5 gives the equation for velocity of liquid in a single closed tube. This is a parabola, with the velocity at the maximum equal in magnitude but opposite in direction to the electrosmotic velocity of the liquid. Curves for cylindrical tube type cells are given by Abramson (1). At depths given by

$$r = \pm\frac{R}{2}\sqrt{2}$$

at which actual electrophoretic velocities are obtained, the velocity gradient (change in velocity with depth) is large; hence inaccurate results are obtained if a slight error in focusing is made.

The same condition is present at the levels used for observation in a single cell of rectangular cross section; in such a cell velocities are obtained at approximately the one-fifth and four-fifths depths, at which depths velocity changes rapidly with depth.

For the new cell the condition was stipulated that there should be no movement of the liquid at the axis of the tube of smaller radius. The velocity of the liquid in this tube expressed as a function of the radius can be obtained from equations 2 and 11; this is

$$v_W = Pr_1^2/4\eta L_1$$

This corresponds to a parabola with the maximum at the points where $r = 0$, and $v_W = 0$. It is evident that the velocity gradient, dv_W/dr_1, is also zero at this point.

The observed velocity of the particles is the algebraic sum of the electrophoretic velocity and the velocity of the liquid. Assuming that the electrophoretic velocity is constant, it is evident that the $V_{\text{obsd.}}$–depth curve is a parabola of the same general form as the v_W–depth curve; the coördinates of the maximum for this curve in the new cell are $r = 0$ and $V_{\text{obsd.}} = U$, and the velocity gradient at this point is zero.

Theoretical advantages of the new cell in comparison with a single tube cell are dependent on the fact that electrophoretic velocities are determined at the maximum of the velocity observed–depth curve. They may be listed as follows: (a) The proper depth for observation is readily found; since observations are made at the depth where the particle velocity is greatest, a few readings above and below this depth aid in checking the correct level. (b) A slight error in focusing does not result in appreciable errors in velocities, since change in velocity with depth is small at points near the correct level. (c) Depth of field of view is not as important a factor as in the case of a single closed tube, in which particles at a slightly different level have noticeably different velocities. (d) Rotational effects on particles are at a minimum, since the velocity gradient near the level of observation is small. This particular advantage suggests the use of a cell of this type for studies on larger particles where the effect of change in velocity with depth is a factor which must be considered.

SUMMARY

1. A new electrophoresis cell for microscopical observations of particle velocities is described; this consists of two tubes in parallel between the electrodes. By extension of the general theory of electrosmotic flow of liquid in tubes, it is shown that the flow of liquid at the one-half depth in the tube of smaller radius can be eliminated if the dimensions of the two tubes have a definite relation to each other. Hence, the observed particle velocities at this depth are the actual electrophoretic velocities, from which the mobilities can be calculated.

2. In this new cell electrophoretic velocities are given at the maximum of the parabolic curve expressing velocity as a function of depth. Advantages resulting thereby are: (a) Difficulty in focusing is alleviated, since the observed velocity is a maximum at the correct level. (b) Slight errors in focusing, or errors due to depth of field of view of the microscope, are less important than in a single tube cell, since the velocity gradient is small near the correct level of observation. (c) Rotational effects of the liquid are less in the case of the new cell, since change of velocity with depth is at a minimum at the depth of observation.

PART II. EXPERIMENTAL COMPARISON OF THE NEW CELL AND A CYLINDRICAL TUBE CELL

As a means of confirming the theory and construction of the new cell, the electrophoretic mobility of quartz particles in distilled water was determined by three different methods, as follows: (a) In the new cell, actual electrophoretic mobilities being determined from observations at the one-half depth in the tube of smaller radius. (b) In a single-tube cell, actual mobilities being obtained from observations at depths where theoretically there should be no movement of the liquid. (c) In a single-tube cell, observing at various depths, and obtaining an approximate value for the electrophoretic mobility.

EXPERIMENTAL PROCEDURE AND RESULTS

General technic

Pieces of quartz tubing (the same material as used in the quartz tubes of the new electrophoresis cell) were cleaned with cleaning solution, thoroughly rinsed with distilled water, then with triply distilled water, dried, and ground in an agate mortar. A suspension of this powder was made in triply distilled water; this was allowed to settle for about four hours, after which the upper portion was siphoned off and used for the experiments. The quartz particles in this portion of the suspension were therefore small enough to stay suspended for some time.

Observation was made in T_1 (see description of the new cell given in Part I), used either as a single-tube cell or in parallel with T_2. The cell was carefully cleaned before using, and thoroughly rinsed, including two rinsings with triply distilled water previous to filling with the suspension. The optical combination consisted of a 10x ocular, a 21x (8 mm. 0.50) objective, and a cardioid condenser giving dark-field illumination. Observations of the velocities were made and mobilities calculated, the potential gradient being given with sufficient accuracy by E/L_1, where E is the applied E.M.F. and L_1 is the length of T_1. Experiments were carried out at room temperature and corrected to a temperature of 25°C. on the assumption that electrophoretic velocity varies inversely as the viscosity of the medium, i.e., $U\eta$ = constant. This would appear probable for suspensions of quartz particles, at least for the small range of temperature (25–29°C.) concerned in the experiments.

Series I

Six experiments were carried out using the new cell. Each experiment consisted of one hundred observations on a suspension of quartz particles prepared as previously described; the current was reversed in order to prevent polarization after each set of five readings, the microscope refocused after twenty readings, and the cell refilled with fresh suspension after sixty readings. The mobilities were calculated, and the arithmetic mean (A.M.) and standard deviation (S.D.) of the values determined; these values were then corrected for a temperature of 25°C.

TABLE 1

Electrophoretic mobilities of quartz particles in distilled water at 25°C. as determined at depths where there was no movement of the liquid in the new cell, and in a single-tube cell

EXPT. NO.	A.M.* AND S.D.† OF MOBILITIES IN μ/SEC./VOLT/CM.			
	Series I—New cell		Series II—Single-tube cell	
	A.M.	S.D.	A.M.	S.D.
1	3.55	0.24	2.84	0.60
2	2.93	0.31	2.76	0.63
3	3.07	0.21	3.56	0.42
4	3.14	0.14	3.00	0.42
5	2.68	0.24	2.83	0.25
6	3.39	0.22	2.73	0.22
Sum..........	18.76		17.72	
A.M...........	3.13		2.95	

* A.M. = arithmetic mean.
† S.D. = standard deviation.

Series II

This series consisted of six experiments, using T_1 as a single-tube cell. The technic followed was the same as in series I, velocities being observed at the depth where there was no movement of the liquid, i.e., where

$$r = \pm \frac{R}{2}\sqrt{2}$$

In the first two experiments of the series, half of the observations were made at the upper level, and half at the lower. In experiments 3, 4, 5, and 6, values were obtained at the upper level only.

Experimental results of series I and II are given in table 1.

Series III

Each of the three experiments in this series consisted of observations taken at levels differing by 50 μ throughout the depth of the cell, from ten to forty observations being taken at each level. From these observations the arithmetic mean of mobilities for each level was calculated. The mobilities at different levels plotted against depth resulted in curves of parabolic form; the mean mobilities of the maxima of these curves, obtained where $r = 0$, were 6.53, 6.24, and 5.61 μ per second per volt per centimeter, respectively, giving a mean mobility for the three experiments of approximately 6.1 μ per second per volt per centimeter. At the walls of the tubes the particles moved in the opposite direction, the mean mobilities for the three experiments giving values of −2.66 and −3.10 μ per second per volt per centimeter at depths of $+R$ and $-R$ respectively.

TABLE 2

Electrophoretic mobilities of quartz particles in distilled water at 25°C., as determined in a single-tube cell from observations obtained at various levels in the cell

EXPT. NO.	MOBILITY IN μ/SEC./VOLT/CM.
1	2.37
2	2.16
3	2.13
Sum..................................	6.66
A.M...................................	2.22

Electrophoretic velocity is given by equation 7. A rough approximation of this velocity is given by

$$U = \frac{2}{R^2} \sum_{r=0}^{r=R} V_{\text{obsd.}} \cdot r \, \mathrm{d}r$$

This approximation is readily seen to be inaccurate unless observations are taken at a large number of levels, since it gives no weight to the value of the velocity at the axis of the tube, where $r = 0$, and gives undue emphasis to the value at the wall of the tube, where $r = R$. A better method of approximation consists in the use of the average of the mobilities observed at depths differing by dr. Values of mobilities so calculated for the three experiments in this series are given in table 2.

DISCUSSION OF RESULTS

Examination of the data in table 1 indicates that practically the same values of mobilities are given in the two cells. The value of the arithmetic mean of the mean mobilities for the six series of experiments of series I is 3.13 μ per second per volt per centimeter with a standard deviation of $\pm$ 0.31 μ per second per volt per centimeter, and for the six experiments of series II, 2.95 $\pm$ 0.31 μ per second per volt per centimeter; hence there is no significant difference in the mobilities, as found in the two cells, greater than that which might be expected from the amount of variation in the experiments comprising a series. The mobility of quartz particles in triply distilled water at 25°C. as obtained by these two methods is therefore approximately 3.0 μ per second per volt per centimeter.

The electrosmotic velocity of the liquid is equal to the difference between the electrophoretic velocity of the particles and the maximum observed velocity of the particles in the single tube (see equation 8); therefore the electrosmotic velocity of the liquid in the present case is $3.0 - 6.1$ or -3.1 μ per second per volt per centimeter. With consideration of the relatively high experimental error inherent in experiments of the type concerned, these results seem to substantiate the theory that the electrophoretic mobility of the particles is equal in magnitude to the electrophoretic mobility of the liquid.

Comparison of mobilities given in table 2 with those given in table 1 shows that mobilities as obtained in the single-tube cell by means of observations taken at various levels in the cell are not equal, within the experimental error, to those obtained in the new cell or in the single-tube cell at depths of $\pm R\sqrt{2}/2$.

A plausible explanation of the different results obtained with the third method would seem to be that values not conforming to the theoretical values are obtained near the walls of the tube. Evidence in direct support of this is disclosed by examination of the data obtained in the three series of experiments. Since the electrosmotic mobility and the electrophoretic mobility appear to be equal in magnitude but opposite in direction, we should expect that there would be no movement of the particles at the wall of the tube. Experimentally, however, the particles at the walls were observed to move in the opposite direction from those at the axis of the tube. On the basis that the velocities should have been zero at the walls of the tube, the experimental values there determined were neglected, and the curves drawn to meet the axis, giving $V_{\text{obsd.}} = 0$ at distances $r = +R$ and $-R$, respectively. By interpolation of the values obtained at distances of 50 μ from the walls, and use of the values as obtained experimentally at the other depths, approximation of mobilities by the method previously employed gave values of 2.87, 2.93, and 3.03 μ per second per volt per centimeter, respectively, for the three experiments. These values are in the range of mobilities as obtained by the other two methods. This method of calculation of mobilities is not to be recommended for general use, however; it depends on the assumption that the electrophoretic mobility of the particles is equal to the electrosmotic mobility of the liquid, a condition which would be expected to occur only in cases where the wall of the tube and the particles are of the same material, and has not been found to occur in all of these cases. Moreover, the method of interpolation does not give accurate results, since it is not known how far the influence of the wall extends. Another objection to the method lies in the large number of observations which must be made to obtain an approximation of the desired accuracy.

The advantages of the new cell in comparison with the single-tube cell, in both cases observations being made at the depths where theoretically there is no movement of the liquid, are apparent from a consideration of the values of standard deviations obtained in the experiments of series I and II; these are given in columns 3 and 5 of table 1. Standard deviation is an index of variance, i.e., it indicates how closely the values obtained are grouped about the arithmetic mean. The standard deviations found for the first two experiments in series II, in which values were observed at both the upper and lower levels in the single-tube cell, are much greater than the values of standard deviations obtained with the new cell. These high values probably came from failure to focus on the correct levels; it is difficult to find the correct levels such that readings at the upper and lower depths agree. It is also difficult to obtain readings at the lower level, therefore readings in the other four experiments of the series were taken at the upper level only. In all cases the microscope was refocused after each set of twenty readings. The values of standard deviations in experiments 3 and 4 of series II are also higher than those for the new cell, probably owing to the readings having been obtained at slightly different levels. In the last two experiments of this series standard deviations are not greater than with the new cell, indicating that observations were made at approximately the same level throughout the experiment; however, since these observations were made only at the upper level, it is difficult to be sure that the correct level was used. The uniformity of the values of standard deviations obtained in the experiments with the new cell (series I) make its advantages apparent. The correct level for observation is obtained readily; moreover, small differences in levels of observation do not introduce large errors in the observed values. For results of a certain degree of accuracy a smaller number of observations is justifiable with the new cell than with a single tube cell.

CONCLUSIONS

1. Values of electrophoretic mobilities of finely ground quartz particles in triply distilled water obtained in the new double-tube cell agreed within the experimental error with values as determined in a single cylindrical tube, in both cases observations being made at depths where theoretically there should be no movement of the liquid.

2. Values of electrosmotic mobilities obtained with a single cylindrical tube from observations made at various levels in the cell did not agree with values as obtained by the other two methods. Experimental evidence suggests that erroneous values are obtained in the walls of the tube.

3. The approximate electrophoretic mobility of quartz particles in triply distilled water as obtained by the two methods which agreed within experimental error was 3.0 μ per second per volt per centimeter.

4. The value of electrosmotic mobility of water in quartz tubes as obtained from these experiments was approximately −3.1 μ per second per volt per centimeter.

5. Consistency of experimental results with the new double-tube cell indicates the advantages of the new cell.

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THE EFFECT OF CERTAIN X-RAYS ON THE ELECTROPHORETIC MOBILITY OF ESCHERICHIA COLI[1]

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Recent researches concerned with the effects of x-rays on bacteria have for the most part considered rates of killing and the theory of action on a sensitive zone. The mechanism of action is not clear, however; certain studies seem to indicate that factors other than direct action on a sensitive zone are involved.

In the elucidation of this problem, changes at the surfaces of the bacteria need to be considered. It is evident that x-rays may affect the distribution of ions at the surface, with a probable resultant change in surface charge and electrokinetic potential. A means of approach to the problem is through the study of electrophoretic mobilities of the particles. Reports of such studies in the literature have been few in number. Lisse and Tittsler (1931) found that irradiation of *Escherichia coli* with ultraviolet light resulted in death with an accompanying decrease in electrophoretic velocity. Dozois *et al.* (1932) reported no change either in viability or in electrophoretic velocity of *Escherichia coli* after irradiation in an aqueous suspension using a Coolidge-type molybdenum-target x-ray tube, the x-rays being filtered only by the lime-glass window of the tube and the wall of the celluloid vessel containing the suspension. As a general rule, x-rays seem to be lethal to bacteria. In apparent contradiction to the absence of lethal effects noted by Dozois *et al.*, Wyckoff (1930) had found that *Escherichia coli* spread on the surface of nutrient agar were killed by direct exposure to x-rays from a molybdenum target, the only filter being the Lindemann glass window of the tube. With these studies in mind and the object of determining whether death through the agency of x-rays was accompanied by a change in the electrophoretic velocity, the procedure determined upon in the present research was to irradiate the bacteria on the surface of nutrient agar, noting the lethal action, and observing any changes in electrophoretic mobilities resulting from such irradiation.

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[1] The data in this paper are taken from a thesis submitted by Margaret E. Smith in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Agricultural and Biological Chemistry, The Pennsylvania State College.

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APPARATUS AND TECHNIQUE

Bacteria. The bacteria used were from a strain of *Escherichia coli* grown on nutrient agar slants. For purposes of experimentation some bacteria were removed from these slants into sterile water and thence to the surface of nutrient agar in Petri dishes, and incubated at 37°C. for 18 to 24 hours. For irradiation the glass cover of the Petri dish was replaced by a lead cover with a hole for the admission of the x-rays. A section of the irradiated portion was used subsequently for the electrophoretic experiments on irradiated bacteria, and a similar section of the part protected by the lead cover was used for the experiments on non-irradiated bacteria, thus ensuring like conditions of growth for the two samples.

X-ray apparatus. Two different x-ray tubes were used: (1) A Coolidge-type water-cooled molybdenum-target tube, having a lime-glass window; (2) a copper target "Phillips Structure Research Tube," the window of this tube being of Lindemann glass. Voltage in each case, supplied by means of alternating current and transformer with the tube itself acting as rectifier, was 30 KV. (root mean square) as determined by a voltmeter-coil on the core of the high voltage transformer; the tube current was maintained at 21 to 22 ma. The distance from the target to the bacteria on the surface of the agar was approximately 13 cm. By means of the two tubes and the additional use of a filter three types of radiation were produced, theoretically of the following quality: (1) Mo characteristic K radiation together with general radiation of wave lengths longer than 0.3 A (approx.), with the exception that the longer wave lengths were cut out to some extent by the lime-glass window of the tube; (2) Cu characteristic K radiation with general radiation longer than 0.3 A.; (3) essentially Cu $K\alpha$ radiation, obtained from the copper target tube with the interposition of a nickel filter approximately 0.5 mil. (0.01 mm.) in thickness and two celluloid filters each approximately 1 mil. (0.02 mm.) in thickness. The intensity of radiation from the molybdenum target tube was not determined; intensities from the copper target tube without and with the filters as measured with an open air ionization chamber[2] placed at the level used for irradiation of bacteria were approximately 950 and 440 *r* per minute respectively.

Suspensions. Bacteria, either from an irradiated or from a non-irradiated section of the agar plate, were removed by a small stream of distilled water from a wash-bottle. The suspension was shaken up with glass beads, filtered through cotton to remove clumps, and diluted with distilled water to the desired density for electrophoretic studies. The suspension was stored in a rubber stoppered Pyrex glass flask in the laboratory until use, the end of the experimentation period being not more than three hours after preparation of the suspension. Determinations of pH were made by the LaMotte method using bromthymol blue as an indicator. Since values so obtained were within the range in which Winslow and Upton (1926) found velocity to be independent of pH, and since no correlation was found in the present studies, data concerning the pH of suspensions are not reported.

Electrophoresis cells. A cell of the Northrop-Kunitz type was used in part of the research; the theory and use of such cells has recently been reviewed by Abramson (1934). After sufficient readings had been made at both the upper and lower "stationary levels" to make certain that the correct depth was found, readings were made only at the upper level. One hundred observations of velocities were obtained for each experiment, reversing the polarity after each set of 5, refocusing the microscope after 20, and refilling the cell with fresh suspension after 40 or 60 readings. The electrophoretic velocity of a particle is customarily recorded as mobility—i.e., velocity per unit potential gradient. Since the cell used did not have the auxiliary electrodes needed to measure the fall of potential through the cell itself, an estimate of this value was obtained from the dimensions of the apparatus. Hence, mobilities as reported with this cell are in reality only approximate mobilities.

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[2] The ionization chamber was kindly loaned by Dr. G. Failla of the Memorial Hospital, New York City.

A new "double tube" cell was developed and used for most of the experimental work; the description, theory and advantages of this cell are presented elsewhere (Smith and Lisse, 1936). Briefly, it consists of two tubes in parallel between the electrodes, the dimensions of the tubes having a relation to each other such that no flow of liquid occurs along the axis of the tube of smaller radius, and therefore velocities at that depth are the actual electrophoretic velocities of the particles. The procedure of reversing polarity, refocusing the microscope and refilling with fresh suspension as used in the work with the Northrop-Kunitz cell was followed. In some cases, however, a total of only 60 or 80 readings were taken in one experiment; a smaller number is permissible, since there is less variation in the readings taken with this cell, due to the fact that observations are made at the level where the velocity gradient is minimum. From these observations values designated as actual mobilities were calculated, the potential gradient through the cell being given with sufficient accuracy by E/L, where E is the fall of potential between the electrodes as measured with a voltmeter, and L is the length of the tube of smaller radius.

RESULTS OF EXPERIMENTS ON LETHAL ACTION OF X-RAYS

A series of experiments were carried out primarily to determine whether the bacteria as used in the electrophoresis studies were killed through the action of x-rays. The technique used in most

of the experiments was similar to that used by Wyckoff (1930), the bacteria being spread on the surface of nutrient agar, a section irradiated, and counts made of the number of colonies developing on an irradiated section and on a control section of equal area. Table 1 gives the results of these experiments, each number being the average of percentages obtained from 10 or more counts from irradiated sections and from an equal number of control sections. Conclusions concerning rates of killing cannot be made from the data, due to insufficient number of experiments and also to the fact that voltage and current were not sufficiently well controlled to allow for quantitative results, particularly in the cases of short-time irradiation. However, allowing for the differences in intensities used, the results are of the same order as those given by Wyckoff.

TABLE 1

Percentage of surviving organisms (Esch. coli) after exposure to x-rays of different nature for various periods of time, from ½ to 120 minutes

PERIOD OF IRRADIATION	TYPE OF RADIATION		
	Mo K characteristic + general	Cu K characteristic + general	Cu K_α
minutes	*per cent*	*per cent*	*per cent*
½		48	60
1		36	41
5		2	5
10	38	0	1
20	12	0	0
120	0	0	0
Intensity in *r* per minute............		950	440

Five experiments were concerned with the lethal effects of copper characteristic K and general radiation on *Escherichia coli* in aqueous suspension. These showed that while the rays were lethal, the rate of killing was much less than when the bacteria were exposed directly. Since it is apparent (see table 1) that x-rays from the molybdenum target are far less lethal than are those from the copper target, an explanation of the absence of lethal effects noted by Dozois *et al.* (1932) may be that insufficient dosages were given to make the effects observable.

Further experiments showed that irradiation of the medium and subsequent covering of the surface of the agar with *Escherichia coli* did not result in death of the bacteria; and also that the lethal effects of copper radiation are cut off, or at least greatly diminished, by the interposition of the glass cover of the Petri dish between the x-rays and the bacteria.

For the electrophoresis studies, the surface of the agar was covered with a more dense suspension than for the above studies; the bacteria were grown for 24 hours at 37°C. before irradiation, thus being in colony form. Sub-cultures were also made from these irradiated sections and incubated to determine the presence of viable cells. Conclusions from the lethal studies which are of particular interest in the electrophoresis studies are:

1. Twenty-minute exposure of *Escherichia coli* to Cu characteristic K and general radiation, or to Cu K_α radiation resulted in death of all of the bacteria in the irradiated section on an agar plate, either in the case where the bacteria were spread out on the plate, or where colony formation was present.
2. Twenty-minute exposure to Mo characteristic K and general radiation did not result in death of all of the bacteria in the irradiated section.
3. Long-time irradiation, with any of the three types of radiation used, resulted in death of all of the bacteria on the irradiated section.

EXPERIMENTAL RESULTS OF ELECTROPHORESIS STUDIES

Five series of experiments concerned with electrophoretic mobilities of irradiated and non-irradiated bacteria were performed. The irradiated bacteria were suspended in distilled water in the manner previously described; conditions of irradiation for the five series were as follows: in series I, the bacteria were exposed for 20 minutes to radiation from the molybdenum target tube, and in series II to unfiltered radiation from the copper target tube for 20 minutes; in series III and IV, the same two types of radiation were used respectively, but the period of exposure was 120 minutes; series V consisted of 240-minute exposure to Cu K_α radiation.

Each series comprised 10 pairs of experiments, the Northrop-Kunitz cell being used for 8 pairs of experiments in series I and 7 pairs in series II, and the new cell for the remainder. A pair of experiments consisted of the 60 to 100 observations on bacteria taken from the irradiated section of the plate, and also of the same number of observations on the non-irradiated bacteria obtained from a protected portion of the same plate. In each pair of experiments, conditions of growth of bacteria, suspension medium, the cell used and temperature were practically identical. The mobility in the case of the new cell, or the approximate mobility in the case of the Northrop-Kunitz cell corresponding to each observed velocity, was calculated, and the mean mobility—*i. e.*, arithmetic mean (A.M.) of the 60 to 100 values was determined.

Frequency distribution diagrams of the mobilities obtained in the experiments indicated that the data for an experiment tend to conform to the normal distribution curve; it appeared that the calculation of standard deviation[3] (S.D.) might be of value. Two advantages of such determinations were made use of in the accumulation of data. The value obtained serves as a criterion for data which need to be examined critically and perhaps discarded, since an unusually high value might result from errors such as focusing on different levels, or drifting in one direction. Furthermore, use of the standard deviation gives information as to the number of values which should be determined in any experiment for the desired amount of accuracy. Since variation in readings with the new cell tended to be less than with the Northrop-Kunitz cell, a smaller number of observations was justified.

Table 2 gives the values of the mean mobilities and the standard deviations calculated for the 10 pairs of experiments in series I.

The per cent change in mobility resulting from irradiation in series I, calculated from the values of the arithmetic means of the

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[3] Standard deviation is defined by:

$$\text{S.D.} = \sqrt{\frac{\Sigma(X - \text{A.M.})^2}{N}}$$

where $X - \text{A.M.}$ is the difference between a value X and the mean, and N is the number of values of X.

mean mobilities for the irradiated and non-irradiated samples (see table 2) is −2.8 per cent. The customary method of treating data of electrophoretic mobilities has been to select a more or less arbitrary per cent change as the limit of experimental error to be allowed. In selecting this value, factors such as the number of experiments performed and the amount of variation of values from the mean should be considered; that is, the use of statistical methods is indicated. In the present study a means of determining whether changes to the extent found are significant was sought through the use of "Student's" method of analysis for paired data. This method is given by Pearson (1914) together with the table of probabilities. Love and Brunson (1924) discuss the applicability of this method, and Love (1924) gives a table

TABLE 2

The mobilities of irradiated and non-irradiated Esch. coli in aqueous solutions

Conditions of irradiation: Mo x-rays from Coolidge type tube operating at 30 $KV_{r.m.s.}$, 22 ma., impinging for 20 minutes on bacteria spread on surface of agar, distance 13 cm.

PAIR NUMBER	CELL USED	A.M. AND S.D. OF MOBILITIES IN μ/SEC./VOLT/CM.			
		Non-irradiated		Irradiated	
		A.M.	S.D.	A.M.	S.D.
(1)	(2)	(3)	(4)	(5)	(6)
1	N.K.*	3.23	0.29	2.96	0.31
2	N.K.	3.62	0.35	3.62	0.32
3	N.K.	3.76	0.32	3.78	0.41
4	N.K.	3.75	0.31	3.53	0.35
5	New†	3.70	0.19	3.95	0.35
6	New	4.03	0.23	3.98	0.30
7	N.K.	3.33	0.27	3.39	0.28
8	N.K.	3.34	0.21	3.28	0.21
9	N.K.	3.33	0.31	2.87	0.23
10	N.K.	3.23	0.29	2.96	0.31
Sum....................		35.32		34.32	
A.M....................		3.53		3.43	

* N.K. = Northrop-Kunitz cell.

† New = new type cell.

of the "odds," indicating whether values determined are significant.

Analysis of the data given in columns (3) and (5) of table 2 with reference to Love's table shows that the odds are less than 12:1 against the difference in mean mobilities of the non-irradiated and irradiated samples being due to chance alone. Odds greater than 20:1 are usually termed significant; odds greater than 100:1 are very significant. Thus, analysis shows that no significant change in electrophoretic mobility was brought about through irradiation of *Escherichia coli* for 20 minutes with x-rays from the molybdenum target tube. A similar analysis of the data given in columns (4) and (6) of table 2 shows that the odds are less than 10:1 against the differences between the standard deviations of the non-irradiated and irradiated samples being due to chance alone; therefore we would conclude that no significant change in the variation of mobilities has resulted from irradiation.

Analyses by "Student's" method of the data obtained in the other four series show that very significant changes in the mobility resulted from irradiation in the experiments of series IV and V, the odds in these 2 cases being greater than 10,000:1 and 300:1 respectively; in no other cases were the changes significant. Table 3 gives a summary of the results obtained in the electrophoretic studies for the five series. Changes which are not of sufficient amount to be significant are indicated in the table by 0; in the 2 cases where significant changes occurred the per cent of

TABLE 3

Summary of changes in mobility and in variation in mobilities of Esch. coli with exposure to x-rays of different wave lengths

SERIES NUMBER	TYPE OF IRRADIATION	INTENSITY	TIME	CHANGE IN MOBILITY	CHANGE IN VARIATION
		r/minute	*minutes*	*per cent*	
I	Mo K + general		20	0*	0
II	Cu K + general	950	20	0	0
III	Mo K + general		120	0	0
IV	Cu K + general	950	120	−15.4	0
V	Cu K_α	440	240	−7.9	0

* 0 indicates that changes were not significant.

change, calculated from the values of the arithmetic means of the mean mobilities, is given.

DISCUSSION OF RESULTS

The lethal studies showed that 20-minute exposure to copper characteristic and general x-radiation resulted in death of all of the bacteria in the irradiated section, whereas exposure for the same length of time to molybdenum characteristic and general radiation resulted in death of only a certain percentage of the bacteria. In either case no significant change in electrophoretic mobility was observed. This clearly indicates that change in electrophoretic mobility is not a criterion of death of the organism, or at least that any change produced is not of sufficient magnitude to be observed by the methods used.

A change in mobility can be brought about through the agency of x-rays as is evidenced by the decreased mobility resulting from long-time irradiation either with copper characteristic and general radiation, or with Cu K_α radiation. It is difficult to state with certainty the cause for the decreased mobility. As noted previously, Lisse and Tittsler (1931) found that irradiation of *Escherichia coli* with ultraviolet light resulted in decreased mobility; it is possible that wave lengths in this range are effective in the present case. Such wave lengths would be present in appreciable amounts in the unfiltered copper radiation, and might result from fluorescent radiation of the Cu K_α radiation, and probably to a very small extent from the molybdenum radiation. Experimental evidence is in accord with this view, since the copper general and characteristic radiation was the most effective, and the molybdenum radiation the least effective in changing the mobilities. Another explanation might be that death of the bacteria brings about conditions, such as changes in permeability or adsorption, which may result after some time in the decrease in mobility noted.

From the studies it appears that there is a difference in action of different wave lengths. The dosages of Cu characteristic and general radiation and of Cu K_α in the experiments of series IV and V were 11.4×10^4 and 10.6×10^4 *r* respectively (see table 3 for data), their ratio being approximately 1.1:1; the ratio of resultant changes in velocity was approximately 15:8. With due consideration of the relatively large experimental error inherent in this type of experiment, it appears that the decrease in mobility is a function of the quality of the radiation, not merely of the intensity. Wyckoff (1932) suggested that the lethal effects of x-rays and ultraviolet light might not be the same, even though semi-logarithmic curves of death were found in both cases. The experiments reported here seem to corroborate this view. Thus, while death through the action of ultraviolet light and certain other agencies may be due to action on the exterior of the organism, the lethal effect of x-rays may be due to direct action on a sensitive zone or nucleus.

It is of interest to note that no change in variation of mobilities occurred, even in the 2 cases where the mean mobilities did change. That is, the values of the mobilities of the irradiated samples seem to conform to distribution curves of the same type as those of the non-irradiated samples.

CONCLUSIONS

1. No significant change in electrophoretic mobility of *Escherichia coli* in aqueous suspension resulted from:

(a) Twenty-minute exposure of the bacteria on the surface of nutrient agar to molybdenum characteristic K and general radiation (30 $KV_{r.m.s.}$, 22 ma., 13 cm. distance); this dosage was sufficient to result in death of only a certain per cent of the organisms in the exposed section.

(b) Twenty-minute exposure of the bacteria to copper characteristic K and general radiation. The dosage of 1.9×10^4 *r* was more than sufficient to result in death of all of the irradiated organisms.

(c) One hundred and twenty-minute exposure of the bacteria to the molybdenum characteristic K and general radiation; this dosage was greater than that required to kill all of the organisms in the exposed section.

2. Death through the agency of characteristic and general molybdenum or copper x-radiation is not accompanied by a

change in the electrophoretic mobility of the bacteria in aqueous suspension to an extent measurable by microscopical studies of the type made.

3. Exposure of *Escherichia coli* on the surface of nutrient agar to dosages of Cu characteristic K and general radiation or to Cu K_α radiation in amounts much greater than is required to bring about death of all of the organisms in the exposed section results in a significant decrease in the electrophoretic mobility of the bacteria in aqueous suspension. The Cu characteristic K and general radiation seems to be more effective in this respect than is the Cu K_α radiation.

4. Values of mobilities of irradiated samples seem to give distribution curves of the same type as those of the non-irradiated samples, no increase in the amount of variation being found as a result of irradiation.

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Fertilizing Pennsylvania Cigar-Leaf Tobacco

By D. E. Haley

Professor of Soil and Phytochemistry, Pennsylvania State College.

Reprinted from

BETTER CROPS WITH PLANT FOOD MAGAZINE

Tobacco plots of the three-year rotation to which fertilizers were applied broadcast in 1934. Left: 500 lbs. 6-8-12, yield 1,470 lbs. per acre. Right: 1,500 lbs. 6-8-12, yield 1,670 lbs. per acre.

Fertilizing Pennsylvania Cigar-leaf Tobacco*

By D. E. Haley

Professor of Soil and Phytochemistry, Pennsylvania State College

LANCASTER county, Pennsylvania, is one of the most prosperous agricultural communities in the United States. There are a number of reasons for this, among which are the inherent fertility of the soil, which is of limestone origin, and the high degree of intelligence manifested by the individual farmers in handling this soil. In no other farming community in the United States is more attention given to the maintenance of soil fertility, and to efficient crop production.

The agricultural background of Lancaster county farmers is an important factor in their success. In the majority of instances, they are direct descendants of the German settlers who first placed the land under cultivation more than 200 years ago, and who themselves represented the twenty-fifth successive generation of farmers in the German Palatinate. It is not unusual to find Lancaster county farms operated at the present time by lineal descendants of the pioneer settlers, never having been transferred to anyone but a member of the same family. Throughout these 200 years, a consistent effort has been made to increase rather than reduce the fertility of these soils, and with considerable success.

The system of agriculture now pursued in Lancaster county is diversified, and the rotation usually includes the raising of cigar-leaf tobacco. The center of production of this tobacco in Pennsylvania is this area. For this reason, research on this crop has been largely confined to this county.

Field experiments on tobacco are conducted on a rather large scale at Roseville, one mile north of the city of Lancaster, under the direction of the Pennsylvania State College in cooperation with the Office of Tobacco Investigations, U. S. Department of Agriculture. The work is supervised by Mr. Otto Olson, the Federal Representative, who is thoroughly familiar with agricultural conditions in the county.

duces a succulent growth, which lowers the resistance of the plant to the invasion of disease organisms. A more resistant plant results when a balance of nitrogen, phosphoric acid, and potash is maintained.

Our plot results show that phosphoric acid is the main limiting factor of plant growth, and this probably holds true for all soils of this area. A poor yield invariably is associated with a low phosphoric acid content of the plants. A high yield is not possible without ample supplies of this element. As in the case of nitrogen, phosphoric acid should be balanced with the other essential plant foods. An excess of phosphoric acid, however, lowers the quality of the plants, especially the burning qualities.

Tobacco plants liberally supplied with potash are better able to withstand drought than plants having less potash, because of the production of cuticle on the surface of the leaves. Plants high in potash appear to offer maximum resistance to the invasion of disease organisms. This is a factor

Tobacco plots of the three-year rotation to wh
Left: 1,000 lbs. 6-8-12, yield 1,974 lbs. per acre.

face and improved texture. Left was fertilized
1,000 lbs. of 6-8-4: right received no fertilizer.

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The prevailing opinion of those in charge of the investigations is that it should conform to the general agricultural system peculiar to the county. This policy prevailed at the Ephrata experimental plots some years ago and is maintained at the Roseville plots. The results obtained from these field experiments, supplemented by laboratory tests and by observations of growing crops of tobacco throughout the county, now point to certain fundamental factors which should be given consideration in the production of tobacco, from the standpoint of both yield and quality. These factors may involve definite changes in farming procedure which, however, will in no way interfere with the maintenance of soil fertility.

Contrary to popular belief of some years ago, a soil does not need to be neutral or alkaline in reaction in order to insure good crop yields. Excellent yields of all of the more common agricultural crops have been obtained from slightly acid soils. Moreover, certain crops seem to be better adapted to an acid rather than to a neutral or alkaline soil; tobacco appears to fall into this general class. Since legumes are common in the rotation system now practiced, a large quantity of available lime must not be present in the soil to insure the growth of the best tobacco. Although legumes are high lime "feeders," excellent yields of legumes have been obtained when the soil was slightly acid in reaction. Some soils, however, are too acid for legumes. In such cases additions of lime in reasonable amounts are to be recommended.

Tobacco plots of the three-year rotation to which fertilizers were applied in the row in 1934. Left: 1,000 lbs. 6-8-12, yield 1,974 lbs. per acre. Right: 1,500 lbs. 6-8-12, yield 2,048 lbs. per acre.

Typical Lancaster county homesteads reflect a degree of agricultural prosperity, due to good soils and good management.

Because the prevailing Lancaster county system is "lime and manure" and because these soils are normally high in lime, they are usually neutral or alkaline in reaction. On the other hand, many famous tobacco soils, such as those in the Connecticut valley, are strongly acid in reaction. During 1935 an experiment was conducted on a farm near Roseville which involved the addition of elemental sulphur to the soil, in conjunction with certain fertilizer mixtures. The quantity of sulphur added was sufficient to increase soil acidity up to and even exceeding the acidity of the average Connecticut tobacco soil. The results indicate that tobacco of good quality may be grown on soils of rather high acidity.

An irrational use of lime is to be avoided on soils where tobacco is grown, for a number of reasons. Phosphorus, the chief limiting factor in plant growth on these soils, is rendered less available to the plant if the soil is alkaline rather than slightly acid in reaction. Furthermore, the absorption of potassium from the soil by the plant is much more efficient under slightly acid than under alkaline conditions. The evidence of one crop, that for 1927, indicates that when the potassium content of the tobacco is high, the lime content is low; and when the potassium content of the tobacco is low, as in the case of an ordinary "dry-year" crop, the lime content is high. When the soil is definitely alkaline in reaction, certain essential elements, such as manganese, may be rendered practically unavailable for plant growth.

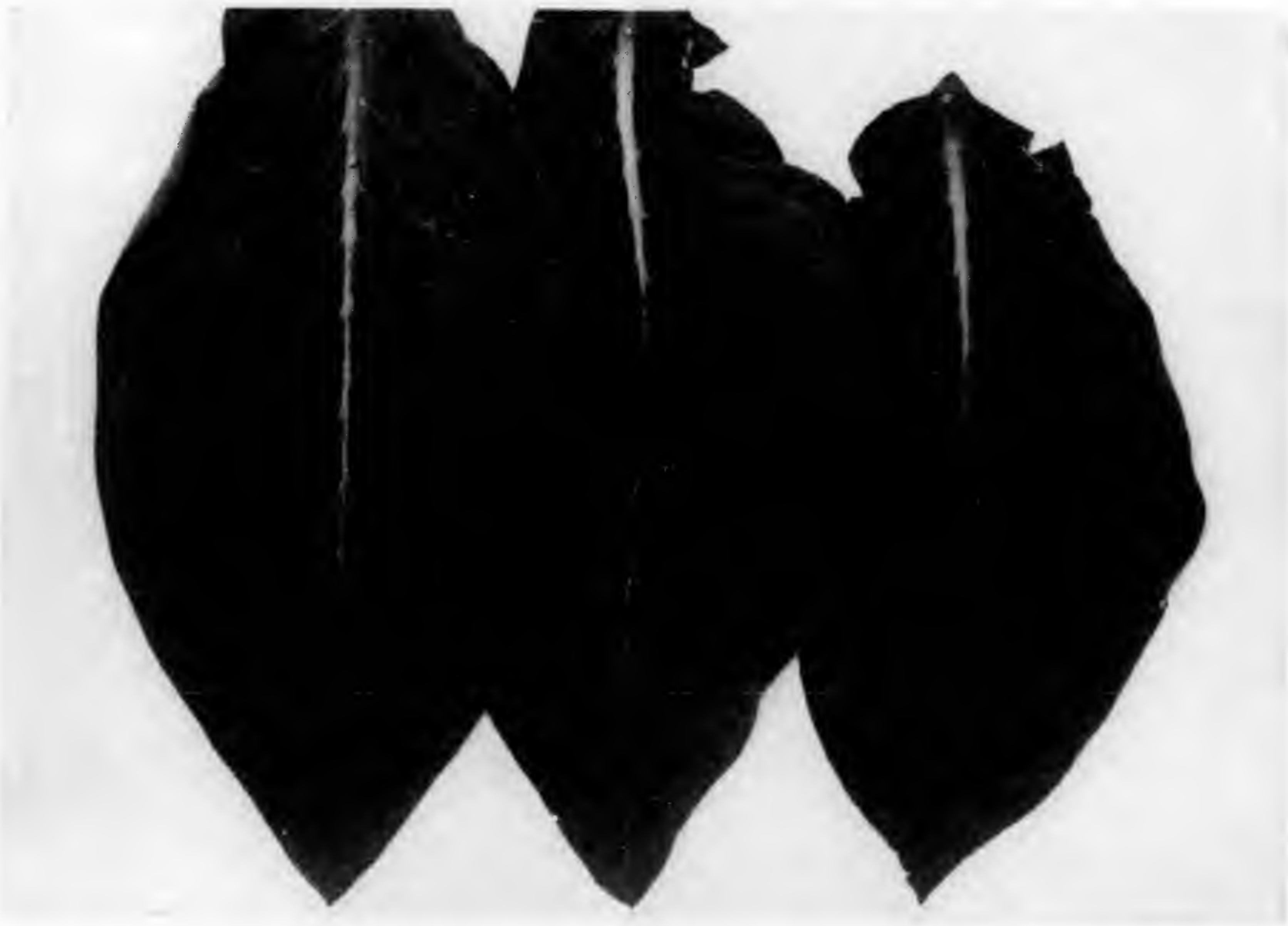
An application of 10 tons of manure per acre, which is the amount ordinarily used by Lancaster county tobacco growers, carries approximately 100 pounds of nitrogen, 50 pounds of phosphoric acid, and 100 pounds of potash. From a percentage standpoint, therefore, it appears to be fairly well balanced with respect to these three constituents; but since the nitrogen is more readily available to the plant than either the phosphoric acid or potash, manure is considered a nitrogen fertilizer as well as a source of organic matter.

The value of manure, from the standpoint of the maintenance of soil fertility, can hardly be overemphasized; it should be used whenever possible. From the standpoint of the most efficient tobacco culture, however, fresh manure should be applied to the crop preceding tobacco in the rotation, or it should be well rotted before being applied just before the land comes into tobacco. If fresh manure is applied directly to the tobacco crop, it should be reinforced with both phosphoric acid and potash.

The tobacco plant requires nitrogen in the early stages of its growth, and a constant supply, within reasonable limits, should be available throughout the growing season. Excellent results have been obtained without the addition of manure, when two-thirds of the nitrogen added was in the form of cottonseed meal and one-third in a readily available mineral form. Beneficial results were not obtained when these proportions were reversed. Heavy additions of nitrogen, without being properly balanced with phosphoric acid and potash, seriously affect the burning quality of tobacco. An unbalanced nitrogen supply also induces a succulent growth, which lowers the resistance of the plant to the invasion of disease organisms. A more resistant plant results when a balance of nitrogen, phosphoric acid, and potash is maintained.

Our plot results show that phosphoric acid is the main limiting factor of plant growth, and this probably holds true for all soils of this area. A poor yield invariably is associated with a low phosphoric acid content of the plants. A high yield is not possible without ample supplies of this element. As in the case of nitrogen, phosphoric acid should be balanced with the other essential plant foods. An excess of phosphoric acid, however, lowers the quality of the plants, especially the burning qualities.

Tobacco plants liberally supplied with potash are better able to withstand drought than plants having less potash, because of the production of cuticle on the surface of the leaves. Plants high in potash appear to offer maximum resistance to the invasion of disease organisms. This is a factor of great importance; in many cases, entire crops have been destroyed by disease. Heavy rains reduce the vitality of the plant, rendering it more susceptible to leaf-spot diseases, especially wildfire, unless the plant is fortified with potash. In addition, no element surpasses potassium in enhancing the burning quality of tobacco.



Potash irons out the leaf, giving smooth surface and improved texture. Left was fertilized with 1,000 lbs. of 6-8-16 per acre; center with 1,000 lbs. of 6-8-4; right received no fertilizer.

The soils of Lancaster county lack available potash. During the season of 1935 crops were observed which showed pronounced symptoms of potash deficiency in the early stages of plant growth, as evidenced by the characteristic mottled appearance of the leaves. Later, an unbalanced condition with respect to nitrogen and potash was observed in many mature plants throughout the county, as evidenced by the characteristic "stem-bound" condition of the leaf. Such a leaf is thicker than a normal leaf and contains more organic matter in proportion to the quantity of potash. This is undesirable from the standpoint of the manufacturer, since it is difficult, if not impossible, to "ferment" smoking qualities into such tobacco.

The problem of potash fertilization is of paramount importance to the tobacco industry of Lancaster county. Notwithstanding the fact that these tobacco soils are deficient in available potash, little potash fertilizer has been used, on some farms none at all. In many cases, fertilizers which are not well balanced with respect to nitrogen, phosphoric acid, and potash have been used. More attention appears to have been given to additions of nitrogen than to additions of either phosphoric acid or potash. This practice must be changed, if the tobacco industry of Lancaster county is to be profitable.

It is difficult, if not impossible, to recommend a fertilizer mixture that will satisfy all conditions in Lancaster county. The field experiments, however, have yielded results which are applicable, in large measure at least, to other parts of the county as well. An application of 1,000 pounds, or more, of commercial fertilizers per acre is desirable in all cases, but the units of nitrogen, phosphoric acid, and potash may vary to some extent on different soils. The nitrogen may vary from 3 to 6 per cent, the phosphoric acid from 5 to 8 per cent, while the potash may vary from 10 to 12 per cent. Potash should be the predominating element. It must be used in a form other than the muriate. The phosphoric acid content should be, on the average, somewhat greater than that of nitrogen.

Excellent results have been obtained on the experiment plot soils, from the standpoint of both yield and quality, when a 6-8-12 mixture has been applied broadcast or to the row in quantities varying from 1,000 to 1,500 pounds per acre, and without manure. Satisfactory results likewise have been obtained on the plots and on different farms located throughout the county when either a 3-5-12 or a 3-8-12 mixture in like quantities has been used in conjunction with stable manure.

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THE VITAMIN CONTENT OF CANNED PINEAPPLE JUICE

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INTRODUCTION

The juices of certain fruits and vegetables have become exceedingly popular as constituents of the human dietary. This increased popularity is apparently due to a combination of qualities or factors, such as palatability, nutritional value, ease of serving, etc. A juice which has received favorable consideration during the past year is that of the pineapple. As to the nutritive value of commercially canned pineapple juice, especially concerning its vitamin content, little can be said, because of the lack of definite scientific data. Data are available, however, which show that both fresh and canned pineapple are good sources of several of the vitamins and, in addition, contain other highly desirable nutritive properties. The investigations of Miller ('24) and Killian ('32) are referred to in this connection.

Since the data presented by the above investigators indicate that both fresh and canned pineapple are good sources of several of the vitamins, it seemed that similar data concerning the vitamin content of commercially canned pineapple juice would have a definite value at this time. The studies herein reported were carried out for the purpose of determining the vitamin A, B, C and G content of this juice.

EXPERIMENTAL

Vitamin A. The method used in the determination of the vitamin A potency of the pineapple juice was essentially that of Sherman and Munsell ('25). Young rats, 20 to 21 days of age and weighing between 40 and 46 gm., were used as the experimental animals. At weaning, each rat was placed in an individual metal cage having a raised screen floor, and was supplied with fresh distilled water and a liberal quantity of the basal (vitamin A-free) diet. An allotment of 0.6 gm. of dry irradiated baker's yeast was fed daily as a source of vitamins B, D and G. All animals were weighed at frequent intervals, at which time a record was made as to the amount of food consumed and the general well-being of the animals. When the animals had been depleted of their body stores of vitamin A, as indicated by cessation of growth and the appearance of incipient xerophthalmia, they were arranged in groups of from six to ten animals, at which point each animal was fed daily an accurately measured quantity of the pineapple juice as the supplement to the vitamin A-free diet. In every case, this supplementary feeding of pineapple juice was continued for a period of at least 6 weeks before the experiments were terminated.

The results obtained through this series of experiments are presented in condensed form in table 1.

The data presented in table 1 indicate that 1 ml. of this juice contains approximately 1 Sherman unit of vitamin A. On the basis of this assay, therefore, the canned pineapple juice tested contained approximately 30 Sherman units of vitamin A per ounce.

Vitamin B (B_1). The Chase and Sherman ('31) method was used in determining the vitamin B potency of the canned pineapple juice. The experimental animals and their care and management were essentially the same as that employed in the vitamin A assays, excepting that a vitamin B deficient diet was employed. When the animals had been depleted of their body reserves, as indicated by cessation of growth and the appearance of mild symptoms of paralysis, they were arranged in groups of from six to ten animals each. A measured amount of the juice to be tested was fed daily in a separate receptacle to each animal. The feeding of the juice was continued for a period of 6 weeks, during which time an accurate record was kept of each animal's weight, the amount of food consumed, and the general appearance of the animal.

TABLE 1

Showing the effect on growth of feeding different daily allotments of pineapple juice to groups of rats which were receiving a diet deficient in vitamin A. For sake of comparison a similar group of rats was fed 1 γ of International carotene daily as a source of vitamin A

DEPLETION PERIOD							SOURCE OF VITAMIN A. QUANTITY FED DAILY	CURATIVE PERIOD						
		Average weight at end of						Average weight at end of						
Group	Average initial weight	7 days	14 days	21 days	28 days	Depletion period		7 days	14 days	21 days	28 days	35 days	42 days	Gain in weight
	gm.	gm.	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	42	64	81	107	123	129	None	127	122	115	. . .	. . .	. . .	. .
2	45	70	86	106	134	138	1.0 ml. of pineapple juice	147	154	159	159	159	161	23
3	44	68	85	100	126	132	1.5 ml. of pineapple juice	142	147	154	158	161	162	30
4	44	68	91	116	130	135	2.0 ml. of pineapple juice	143	149	158	163	165	169	34
5	45	67	89	111	131	134	3.0 ml. of pineapple juice	143	150	159	165	169	176	42
6	44	68	87	116	131	138	4.0 ml. of pineapple juice	149	161	172	181	191	196	58
7	45	67	93	114	132	136	5.0 ml. of pineapple juice	143	155	169	180	187	198	62
8	41	61	80	103	118	123	1 γ International carotene	130	139	143	149	153	158	35

The results obtained in this series of experiments are presented in condensed form in table 2.

From these results it is evident that 1.5 ml. of this canned pineapple juice contained at least 1 Sherman unit of vitamin B. On this basis, 1 ounce of this juice contained approximately 20 Sherman units of this vitamin.

Vitamin G (B_2). The method used in determining the vitamin G potency of the canned pineapple juice was that described by Bourquin and Sherman ('31). Here again young rats, 20 to 21 days of age and weighing between 40 and 46 gm., were used as the experimental animals. The care and treatment of the experimental animals was the same as that reported under vitamin A. After the test animals had been partially depleted of their body stores of vitamin G, as indicated by a definite decrease in their growth rate, they were arranged in groups of from six to ten animals per group and were fed daily measured quantities of the pineapple juice. The feeding of this juice to groups of rats as a supplement to a vitamin G deficient diet was continued for a period of 8 weeks, during which all observations were accurately recorded.

TABLE 2

Showing the growth stimulating effect of pineapple juice when fed at different levels to groups of rats which were receiving a diet deficient in vitamin B. For sake of comparison a similar group of rats received 10 mg. of an International vitamin B preparation

DEPLETION PERIOD					SOURCE OF VITAMIN B. QUANTITY FED DAILY	CURATIVE PERIOD						
Group	Average initial weight	Average weight at end of				Average weight at end of						Gain in weight
		7 days	14 days	Depletion period		7 days	14 days	21 days	28 days	35 days	42 days	
	gm.	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	42	53	58	64	None	60	49	40	..	..	..	..
2	45	59	64	66	1.0 ml. of pineapple juice	68	69	70	72	73	70	4
3	42	56	63	62	1.5 ml. of pineapple juice	68	71	73	75	81	85	23
4	45	55	65	64	2.0 ml. of pineapple juice	67	74	80	85	89	90	26
5	42	52	63	60	3.0 ml. of pineapple juice	64	76	82	84	92	96	36
6	43	53	63	64	4.0 ml. of pineapple juice	71	83	95	101	114	118	54
7	41	49	62	61	10 mg. of International vitamin B preparation	72	77	84	91	98	102	41

The results obtained by this series of experiments are presented in table 3.

The data presented above indicate that a daily allotment of 12 ml. of the canned pineapple juice furnished slightly more than 1 Sherman unit of this vitamin. According to this assay, each ounce of this juice contained approximately 2.5 Sherman units of vitamin G.

TABLE 3

Showing the growth stimulating effect of pineapple juice when fed at different levels to groups of rats which were receiving a diet deficient in vitamin G.

DEPLETION PERIOD					SOURCE OF VITAMIN G. QUANTITY FED DAILY	CURATIVE PERIOD								
Group	Average initial weight	Average weight at end of				Average weight at end of								Gain in weight
		7 days	14 days	Depletion period		7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	
	gm.	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	43	47	53	54	None	56	58	55	53	51	51	49	46	—8
2	42	49	53	53	4.0 ml. of pineapple juice	55	55	56	56	59	59	55	56	3
3	44	53	57	57	6.0 ml. of pineapple juice	63	63	65	64	65	64	63	62	5
4	42	50	52	51	8.0 ml. of pineapple juice	56	54	54	55	55	55	54	55	4
5	44	53	55	56	10.0 ml. of pineapple juice	63	65	64	66	66	68	65	66	10
6	44	51	54	53	12.0 ml. of pineapple juice	62	65	67	68	73	74	76	77	24
7	42	49	53	52	14.0 ml. of pineapple juice	60	64	66	67	69	71	76	81	29

Vitamin C. The vitamin C content of the canned pineapple juice was determined according to the prophylactic technic of Sherman, La Mer and Campbell ('22). Young guinea pigs, ranging in weight from 250 to 300 gm. each, were kept in individual metal cages, which were provided with raised screen bottoms. These animals were arranged in groups of from six to ten individuals per group, and each guinea pig was provided with a weighed but liberal quantity of the scurvy producing diet. Fresh distilled water was provided daily for all animals. Each animal was weighed at weekly intervals and an accurate record was made of the amount of food consumed. At each weighing period, all residual food remaining from the previous week was discarded and a freshly prepared portion fed.

A supply of the pineapple juice sufficient for 1 week was kept in the refrigerator at all times, and the cans were opened just prior to the feeding of the juice. Starting with the first day of the experimental period, an accurately measured amount of pineapple juice, in a clean earthenware container, was fed daily to each pig. The animals comprising each of the several groups received identical allotments of the juice under test. The duration of the test period ranged from 56 to 90 days. When it became definitely evident that certain groups of pigs were receiving pineapple juice in great excess of that required to protect them against scurvy, such groups of animals were discontinued. Those receiving the higher levels of juice (10 ml. daily) were discontinued after 56 days, while those that received slightly less juice (9 ml. daily) were discontinued at the end of 70 days. All animals that received less than a protective dose of the juice and those that received a protective dose or slightly more than a protective dose were continued on experiment until death ensued or throughout the 90-day period. All animals that died and those that were killed at the termination of the various experiments were autopsied and rated according to the various degrees of scurvy manifested (Sherman, La Mer and Campbell, '22). These various degrees of scurvy (viz., hemorrhage of rib junction, muscles, joints, and intestines; fragility of the jaw bone and of the joints; looseness of the teeth; beading of the rib junctions) were scored from 0 to 3, according to the degree of scurvy manifested by each tissue. According to this method of scoring, a score of 8 indicated definite scurvy, while a score of 24 indicated the highest possible degree of scurvy. The quantity of juice required to produce a score of from 0 to 2 was considered the minimum protective dose.

The data obtained from this series of experiments are presented in table 4. It will be noted that all animals of groups 1 and 2 died during the course of the experiment. It may be of interest to state in this connection that all animals of group 1 were dead by the thirty-first day, while all animals of group 2 were dead by the forty-second day.

From the data presented in table 4, it is evident that canned pineapple juice contains appreciable quantities of vitamin C. Approximately 7 ml. of this juice were required to furnish a minimum protective dose of this vitamin. From this, it follows that the canned pineapple juice tested contained approximately four minimum protective doses of vitamin C per ounce. Since a minimum protective dose of vitamin C is approximately 10 International units of this vitamin, it would follow from the above scurvy scores that 1 ounce of canned pineapple juice contained approximately 40 International units of vitamin C.

TABLE 4

Showing the results obtained by feeding varying amounts of canned pineapple juice to groups of young guinea pigs that were receiving a scurvy-producing diet

ANIMAL GROUP NO.	NUMBER OF ANIMALS CONSIDERED	QUANTITY OF JUICE FED DAILY	AVERAGE DAILY GAIN IN WEIGHT	AVERAGE SCURVY SCORE
		ml.	*gm.*	
1	6	0	All died	Severe
2	6	3.0	All died	Severe
3	3	4.0	0	17
4	4	5.0	0.5	8
5	9	6.0	1.8	3
6	7	7.0	2.4	0–2
7	5	8.0	2.7	0
8	8	9.0	2.7	0
9	7	10.0	2.9	0

SUMMARY

A biological assay of canned pineapple juice for its vitamin content, using standard biological methods, showed this juice to be a good source of vitamins A and B, a fair source of vitamin C, and to contain a measurable quantity of vitamin G. When expressed in terms of vitamin units, 1 ounce of this juice contained approximately 30 Sherman units of vitamin A, 20 Sherman units of vitamin B, 2.5 Sherman units of vitamin G, and four minimum protective doses or 40 International units of vitamin C.

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Leaflet 44 April, 1936

FEEDING CHICKS

J. E. Hunter, H. C. Knandel, and John Vandervort

A good ration for poultry is composed of six different classes of materials: proteins, carbonhydrates, fats, minerals, vitamins, and water.

Proteins and Their Value.— Proteins are perhaps the most costly ingredients of poultry feeds. Chicks use proteins for growth, replacing of worn-out tissues and the formation of feathers. Proteins are made up of simpler substances known as amino acids of which there are 20 or more. Since all protein-carrying materials do not contain all of the necessary amino acids, it is advisable to include a variety of protein carriers, both animal and vegetable, in the ration. A ration containing a variety of animal and vegetable protein sources is more likely to be adequate for maximum nutrition than a ration made up of only one vegetable and one animal protein source.

Carbohydrates and Fats.—Carbohydrates and fats are burned in the body to supply fuel and energy. Carbohydrates include starches, sugars, and fiber. The fiber in the ration is indigestible but is generally believed to be beneficial if not present in excessive amounts. Fats are usually supplied to poultry in small amounts because they are digested with difficulty and absorbed slowly.

Minerals.— Minerals are found in all portions of the bird's body, although concentrated in the skeleton. The bird requires calcium, phosphorus, magnesium, iron, sulphur, sodium, potassium, iodine, copper, and possibly others. Generally, all of these elements with the exception of calcium and possibly phosphorus are supplied by the ingredients commonly used in a poultry ration. It is generally agreed that a ration for growing chicks should carry about twice as much calcium as phosphorus. In general, chick rations in which the common sources of protein are used contain about .75 per cent phosphorus. The calcium content of such rations should be approximately 1.5 per cent. This does not mean that 1.5 per cent of calcium carriers should be added to the ration because the ingredients making up the ration supply some calcium. Enough calcium carbonate should be added to the mash to balance the phosphorus present. After the birds are four weeks of age and have started eating scratch grain, additional calcium in the form of oyster shell, limestone grit, or some other good source of calcium carbonate should be kept in hoppers before the birds at all times. When this supplement is first supplied, it may be scattered in small quantities on top of the mash.

Danger in Excessive Quantities of Calcium and Phosphorus.— Ample quantities of calcium and phosphorus must be present in the ration to enable the birds to build strong, healthy bones. Excessive quantities of these elements are likely to lead to the occurrence of hock disease which is sometimes known as slipped tendons or perosis. Chicks reared on wire floors or in batteries are particularly susceptible to hock disease. The supply of calcium carbonate in hoppers will not cause this difficulty because the birds will not eat more than they need. The addition of bone meal to the mash in excess

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quantities or the use of a very low protein meat scrap which is high in bone may lead to the development of hock disease.

The Importance of Vitamins.—Poultrymen realize the importance of vitamins in chick rations and an effort should be made to select ingredients that are high in vitamin value. The commonly accepted vitamins are designated by the letters of the alphabet A, B, C, D, E, and G. Various deficiency diseases result when certain vitamins are lacking.

A deficiency of vitamin A may result in an eye disorder and the condition may be accompanied by pus pockets appearing in the roof of the mouth and the esophagus. The kidneys may appear very light in color because of deposits of urates. Vitamin A is supplied in practical feeding by the use of liberal quantities of yellow corn, by alfalfa or other dried green material, by fresh green feed, and by cod liver oil.

A deficiency of vitamin B leads to loss of appetite, emaciation, convulsions, and death. Since this factor is found in abundance in cereal grains, this disease is not common but might result if large quantities of degerminated cereal products were used.

A deficiency disease resulting from a lack of vitamin C in the ration is not likely to occur.

Vitamin D not Furnished by Ordinary Ingredients.—If chicks are deprived of sunshine and no source of vitamin D is added to the ration, a deficiency disease called rickets, in which the bones fail to harden properly, is likely to occur. Vitamin D is necessary to enable chicks to absorb and use the bone-forming minerals from the digestive tract. Since the common ingredients of a chick ration do not supply vitamin D, this factor must be added separately. Cod liver oil, sardine oil, and some other fish oils are sources of this essential.

Potency of Vitamin D Supplements Expressed in Units.— The potency of vitamin D oils is commonly expressed as U. S. P. units per gram. A good grade of oil usually contains about 100 U. S. P. units per gram or 45,400 units per pound. Fortified or concentrated oils usually contain from three to four times as many units as straight oils. It is usually possible to obtain the potency of an oil expressed in U. S. P. units from the manufacturer or the sales agency marketing the oil.

Vitamin D Requirements of Chicks.— The Pennsylvania State College has shown that growing chicks when deprived of sunlight require approximately 17,500 units of vitamin D per 100 pounds of all mash feed consumed. If an equal proportion of grain and mash is consumed, the above figure should be doubled in the mash provided all of the vitamin D is to be added to the mash. Under these conditions, the mash should contain approximately 35,000 U. S. P. units of vitamin D per 100 pounds. Since an ordinary oil contains approximately 45,400 units per pound, roughly three-fourths pound of such oil would be needed in 100 pounds of mash when scratch and mash are fed in equal amounts. Many poultrymen that mix their feed at home do not have scales to weigh small quantities, and prefer to measure the oil instead of weighing it. A pint of oil weighs approximately one pound.

Smaller Amounts of Vitamin D May Be Used.—If the chicks have access to sunlight through open front houses or if they are on range, it is not so necessary to supply vitamin D. It is felt, however, that it is desirable to feed some vitamin D but if the chicks have access to direct sunlight, it is perfectly safe to cut the above recommendations in half. Sunlight transmitted through ordinary window glass is not effective as a source of vitamin D.

Ordinary rations will supply all of the vitamin E known to be required by chicks.

Vitamin G is an important vitamin for growing chicks and a deficiency of this vitamin will lead to a nutritional disease called pellagra. Milk products, alfalfa, and fish meal contain liberal quantities of this factor and if used in ample quantities will prevent the occurrence of any trouble from the lack of this vitamin.

Ration Designed to Meet Known Requirements of Chicks.—The following ration which has been used for a number of years on the college farm has proved satisfactory and under ordinary conditions should prevent the occurrence of any of the deficiency diseases discussed above. High quality ingredients should be selected when making up the ration.

Ration and Suggestions for Feeding Chicks

Mash

350 lbs.	ground yellow corn
150 lbs.	wheat bran
150 lbs.	wheat middlings
100 lbs.	ground heavy oats
50 lbs.	meat scrap*
50 lbs.	fish meal*
75 lbs.	dried milk
75 lbs.	alfalfa meal
10 lbs.	calcium carbonate
10 lbs.	cod liver oil or sardine oil
5 lbs.	salt
1025 lbs.	total

Grain

100 lbs.	medium cracked corn
100 lbs.	wheat

Barley, oats or buckwheat may be added to the grain mixture in an amount not to exceed 25% of the total grain mixture.

*In place of 50 pounds of meat scrap and 50 pounds of fish meal, 100 pounds of either meat scrap or fish meal may be used.

Feeding Directions.—As a rule, chicks should be fed when well hardened off and ready to be placed in the brooder house. Chicks shipped from a distance are ready to be fed soon after arrival. Holding chicks longer than 72 hours may prove detrimental in that they are actually in a weakened condition. If, for some reason, it is necessary to hold the chicks without feed for several hours after removal from the incubator they can be kept in chick boxes in a room sufficiently farm for human occupancy.

Mash feed should be ready for them when placed on the brooder house floor. Picking appears to be a natural instinct and if feed is not available, chicks may acquire the habit of picking at litter or droppings. Then, too, there is the possibility they may start to pick at each other.

Limited amounts of chick sized scratch grain placed on top of the mash may be fed as soon as the chicks are placed in the brooder house. After the chicks have learned to eat the grain it should be supplied in separate hoppers. After the chicks are eight weeks of age a coarse scratch grain may be fed. When the chicks are 12 weeks old the dried milk in the mash formula should be reduced to 50 pounds. If whole corn is to be fed to the mature birds, it is a good practice to start feeding it to the chicks at about 12 weeks of age.

Feed Containers Should Aid Sanitation.—To prevent a tremendous waste of chick mash a non-wasting hopper with a reel should be used. Overfilling hoppers is another practice resulting in waste. Aside from the loss of feed there is more danger from spread of disease if chicks pick mash from an unclean floor. Placing all hoppers on low wire frames will prevent chicks from eating feed which has spilled from the hoppers and a similar practice for water fountains will keep chicks away from damp areas.

There are many types of troughs available for feeding young chicks. Naturally, the first feeders should be of such a height as to permit chicks to see the feed and to secure it without effort. In view of the fact that disease infection may be carried through the droppings, it would seem desirable to provide a type of hopper which would prevent chicks from getting into their feed and leaving droppings there. A reel across the top of the hopper will accomplish this. As the chicks grow older, larger hoppers can be supplied. Enough hoppers should be supplied so that the chicks will not crowd when eating. Plans of hoppers for two ages of chicks are contained in Leaflet 42.

Increasing Grain Consumption.—Difficulty may be experienced in getting pullets to consume liberal quantities of grain when they are nearly half grown. A method of accomplishing this is to keep both grain and mash before the birds at all times. Another method consists of closing the mash hoppers about 3 o'clock in the afternoon. The chicks are then ready for a liberal quantity of grain late in the afternoon.

Clean Fresh Water Essential.—Water is essential to the life of the chick. It should be supplied continuously from the time the chicks are given their first feed. For very young chicks during cold weather it would be a good practice to remove the chill from the water. A sufficient number of water containers should be provided to prevent crowding while drinking.

Growth and Feed Consumption.—The following tables give growth and feed consumption for Single Comb White Leghorn and Barred Plymouth Rock chicks at various ages:

Weight in Pounds per 100 Chicks

Single Comb White Leghorns

Sex	*Hatching*	*3 Weeks*	*8 Weeks*	*16 Weeks*
Males	8.0141	34.370	135.545	
Females	7.995	31.846	114.550	235.694
		Barred Plymouth Rocks		
Males	8.443	38.201	153.241	
Females	8.350	35.498	132.763	301.415

Feed Consumption per 100 Chicks

Single Comb White Leghorns

Age in weeks	*Lbs. of mash*	*Lbs. of mash to date*	*Lbs. of grain*	*Lbs. of grain to date*	*Lbs. of mash and grain*	*Lbs. of mash and grain to date*
1-4	116.50	116.50	....	....	116.50	116.50
5-8	276.83	393.33	....	....	276.83	393.33
9-12	236.91	630.24	97.34	97.34	334.25	727.58
13-16	213.06	843.30	156.67	254.01	369.73	1097.31
17-20	266.26	1109.56	141.50	395.51	407.76	1505.07
21-24	287.73	1397.29	175.05	570.56	462.78	1967.85
			Barred Plymouth Rocks			
1-4	110.06	110.06	....	....	110.06	110.06
5-8	314.78	424.84	....	....	314.78	424.84
9-12	295.14	719.98	93.53	93.53	388.67	813.51
13-16	263.71	983.69	161.62	255.15	425.33	1238.84
17-20	304.05	1287.74	191.54	446.69	495.59	1734.43
21-24	258.06	1545.80	285.50	732.19	543.56	2277.99

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ACCURACY OF THE DETERMINATION OF LEAD AND ARSENIC ON APPLES

BY

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(Contribution from Pennsylvania State Agricultural College)

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ACCURACY OF THE DETERMINATION OF LEAD AND ARSENIC ON APPLES[1]

By DONALD E. H. FREAR and W. S. HODGKISS, *Department of Agricultural and Biological Chemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

The accuracy of the Gutzeit method for the determination of small amounts of arsenic has been studied by serveral workers (*2, 3, 4, 8*),[2] but the accuracy of the determination of similar small amounts of lead has apparently not been investigated. Federal and State regulatory bodies have now concerned themselves with the amount of lead present on edible fruit as spray residue and have set up limits which legally may not be exceeded.

The legal limits for lead and arsenic trioxide during the season of 1935 have been set at 0.018 and 0.010 grain per pound, respectively. In view of these limits, and the fact that the limits for lead are being gradually reduced, the accuracy of the determinations of these elements becomes important to the chemist, for the analytical results must be interpreted in relation to these legal tolerances.

Any study of the accuracy of these determinations naturally divides itself into two parts: The accuracy of the sampling, and the accuracy of the determination itself. The summation of the errors in these two phases of the work represent the errors in the final determination.

Lead, when present on the surface of apples as spray residue, may combine chemically with the waxy coating of the fruit (*5*), and thus it presents a different problem from arsenic, which apparently does not so combine. In this study the accuracy of the chemical determination of lead will be discussed first, followed by a discussion of the accuracy of the estimation as a whole (including sampling) of both lead and arsenic. The accuracy of the chemical determination of arsenic by the Gutzeit method has been discussed at length (*4, 7*), and will not be considered here.

ACCURACY OF THE CHEMICAL DETERMINATION OF LEAD BY THE PHOTOELECTRIC METHOD

The photoelectric method for the determination of lead on fruit, proposed by Frear and Haley (*6*), is briefly described in order that its advantages and limitations may be better understood.

Light from an electric bulb, regulated by a suitable rheostat, is allowed to shine upon the surface of a photronic cell (Weston no. 594), which is connected directly to a microammeter having a capacity of 200 microamperes. Between the source of light and the photronic cell is interposed a cylindrical glass tube containing the solution to be analyzed for lead. The reading of the microammeter is brought to the maximum of the instrument through an adjustment of the intensity of the light by means of the rheostat. To the solution in the tube is then added sufficient sodium sulphide to precipitate completely the lead present as colloidal lead sulphide. The brown colloidal precipitate reduces the amount of light passing through the solution and falling upon the surface of the photronic cell. The relation between the amount of light transmitted, as measured by the microammeter connected with the cell, and the amount of lead present may be easily determined by standardization with known amounts of lead.

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[1] Received for publication Nov. 13, 1935; issued May 1936. Technical Paper No. 647 of the Pennsylvania Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 644.

It is apparent that two sources of error are inherent in the method: The sum of the errors due to variation in technique, reagents, etc., which may be called the error of operation; and the error of standardization.

To determine the probable error of a single or of the mean of duplicate determinations at a level which will fall within the range of practical work, 50 determinations were made on solutions each containing 0.0004 g of lead as lead acetate (table 1). These solutions were each carried through the entire analytical procedure, including the digestion with concentrated sulphuric and nitric acids, which is used on samples of apple wash to destroy the organic material. The quantity of lead was selected at 0.0004 g since this is equivalent to a concentration of 0.020 grain of lead per pound of apples when an aliquot of the apple wash representing 140 g of apples is taken for analysis.

Table 1.—*Analyses of 50 aliquot portions of solution, each of which contained 0.4000 mg of lead*

Sample no.	Microammeter reading	Milligrams of lead	Sample no.	Microammeter reading	Milligrams of lead	Sample no.	Microammeter reading	Milligrams of lead
1	54	0.400	18	56	0.393	35	53	0.404
2	52	.410	19	53	.404	36	55	.396
3	52	.410	20	54	.400	37	54	.400
4	54	.400	21	52	.410	38	54	.400
5	55	.396	22	56	.393	39	54	.400
6	56	.393	23	57	.390	40	55	.396
7	53	.404	24	53	.404	41	54	.400
8	53	.404	25	53	.404	42	53	.404
9	53	.404	26	54	.400	43	55	.396
10	54	.400	27	52	.410	44	54	.400
11	55	.396	28	56	.393	45	56	.393
12	55	.396	29	56	.393	46	53	.404
13	54	.400	30	53	.404	47	52	.410
14	55	.396	31	52	.410	48	55	.396
15	56	.393	32	55	.396	49	54	.400
16	55	.396	33	54	.400	50	53	.404
17	56	.393	34	55	.396			

By the use of the formula $P.\ E. = \frac{0.6745\sigma}{\sqrt{n}}$, in which the standard deviation is calculated by the formula $\Sigma = \sqrt{\frac{D^2}{n}}$, the probable error of the microammeter readings obtained by analyzing the 50 samples was calculated. The mean value was 54±0.13 microamperes, with a probable error of a single determination of ±0.89 microampere, and of the mean of duplicate determinations of ±0.63 microampere. Since with the instrument used it was not possible to make readings more closely than 1 microampere, the probable error of even a single determination was less than a measurable value.

To translate the microammeter readings into terms of lead concentration, a standardization curve was used which had been constructed by using the mean of 10 determinations at 5 different concentrations. The microammeter reading on this curve corresponding to 0.0004 g of lead was 54 microamperes. By the same formulae as above, the calculated probable error of a single determination was ±0.0036 mg; and that of the mean of duplicate determinations was ±0.0026 mg.

The probable error of the determination as a whole is further affected, by definition, by any error in the preparation of the curve of standardization. To include this factor, the formula $P.\ E. = \sqrt{(P.\ E._s)^2 + (P.\ E._{10})^2}$, in which $P.\ E.$ is the probable error of the method as a whole, $P.\ E._s$ is the probable error of a single determination, and $P.\ E._{10}$ is the probable error of the 10 determinations made to establish the point of reference on the curve of standardization. When the calculation is carried out, the true probable error of a single determination is ±0.0038 mg. When the probable error of duplicate determinations is calculated, the value is ±0.0028 mg.

A comparison of the relative accuracy of the lead method with that of the modified Gutzeit method for the determination of arsenic studied by Barnes and Murray (*4*) indicates that when the quantities determined are considered the method for lead exhibits a relatively higher accuracy than the method for arsenic. Barnes and Murray state that with a solution containing 0.0273 mg of As_2O_3, the probable error of duplicate determinations was ±0.0023 mg and, when the probable error of standardization was considered, the true probable error of the determination was ±0.0025 mg, the mean of 10 individuals being used to locate each point on the standardization curve. The corresponding figures found for the lead determination, in which the standard solution contained 0.4000 mg, calculated in the same manner, were respectively ±0.0026 mg and ±0.0028 mg.

ACCURACY OF THE FINAL RESULTS FROM THE ANALYSIS OF LEAD AND ARSENIC

To include the errors due to sampling, which obviously affect the accuracy of the final values for lead and arsenic, the procedure described below was followed.

Samples of apples, ranging in weight from approximately 7 to 20 kg, were selected, usually picked from the lower limbs of trees in the orchards. Ordinary precautions were taken in handling, and apples of nearly the same size were selected. These samples were taken to the laboratory, and from them two lots of 700 g each were taken, and each washed thoroughly with boiling 1.5-percent hydrochloric acid with 1.0 percent of sodium chloride added. The washings, when cool, were made up to 500 ml with acid of the same concentration. The lead was determined on an aliquot by the method described above (*6*), and arsenic was determined by the Gutzeit method as described in the Official Methods (*1*). In all, 164 lots were analyzed, with a wide range of lead and arsenic content. The results are given in table 2. For convenience, the first of the duplicate lots is designated as A, the second B.

The mean As_2O_3 content of the A samples was 0.00950 grain per pound, and of the B samples 0.00938 grain per pound. The mean of the average of the A and B samples was 0.00944 grain, and the average deviation was 0.00070 grain, or 7.4 percent of the mean. For lead, the mean of the A samples was 0.02255 grain per pound, and for the B samples 0.02235 grain, while the mean of the averages of the A and B samples was 0.02246 grain per pound. The average deviation was 0.00152 grain of lead per pound, or 6.8 percent of the mean.

TABLE 2.—*Arsenic and lead (grain per pound)* [1] *found in duplicate samples of apples*

Sample	Arsenic as As_2O_3				Lead			
	A	B	Mean	Deviation	A	B	Mean	Deviation
B2	0.001	0.001	0.0010	0.0000	0.003	0.005	0.0040	0.0010
B17	.006	.007	.0065	.0005	.016	.018	.0170	.0010
B20	.016	.016	.0160	.0000	.028	.028	.0280	.0000
B21	.010	.011	.0105	.0005	.022	.026	.0240	.0020
B23	.001	.001	.0010	.0000	.003	.003	.0030	.0000
B25	.002	.002	.0020	.0000	.007	.008	.0075	.0005
B26	.012	.009	.0105	.0015	.028	.024	.0260	.0020
B27	.002	.002	.0020	.0000	.005	.013	.0090	.0040
B29	.008	.006	.0070	.0010	.022	.016	.0190	.0030
B30	.012	.010	.0110	.0010	.029	.027	.0280	.0010
B33	.018	.018	.0180	.0000	.033	.034	.0335	.0005
B34	.010	.012	.0110	.0010	.026	.026	.0260	.0000
B40	.005	.005	.0050	.0000	.013	.010	.0115	.0015
B46	.032	.032	.0320	.0000	.067	.077	.0720	.0050
B47	.042	.042	.0420	.0000	.082	.080	.0810	.0010
B48	.015	.016	.0155	.0005	.035	.039	.0370	.0020
B49	.007	.006	.0065	.0005	.013	.010	.0115	.0015
B51	.026	.024	.0250	.0010	.046	.055	.0505	.0045
B52	.007	.008	.0075	.0005	.021	.022	.0215	.0005
B59	.002	.002	.0020	.0000	.006	.008	.0070	.0010
B139	.006	.005	.0055	.0005	.018	.015	.0165	.0015
B146	.006	.006	.0060	.0000	.013	.016	.0145	.0015
B148	.011	.013	.0120	.0010	.032	.035	.0335	.0015
B149	.009	.011	.0100	.0010	.022	.020	.0210	.0010
B150	.012	.012	.0120	.0000	.035	.026	.0305	.0045
B152	.009	.008	.0085	.0005	.037	.025	.0310	.0060
B155	.011	.011	.0110	.0000	.040	.038	.0390	.0010
B224	.003	.002	.0025	.0005	.006	.004	.0050	.0010
B228	.001	.001	.0010	.0000	.003	.002	.0025	.0005
B230	.001	.002	.0015	.0005	.003	.005	.0040	.0010
B231	.002	.003	.0025	.0005	.005	.009	.0070	.0020
B232	.005	.005	.0050	.0000	.012	.007	.0095	.0025
B233	.005	.005	.0050	.0000	.012	.015	.0135	.0015
B234	.003	.002	.0025	.0005	.006	.004	.0050	.0010
B238	.011	.012	.0115	.0005	.028	.032	.0300	.0020
B239	.007	.004	.0055	.0015	.010	.008	.0090	.0010
A36	.005	.004	.0045	.0005	.011	.013	.0120	.0010
A37	.007	.006	.0065	.0005	.021	.019	.0200	.0010
A38	.005	.005	.0050	.0000	.010	.014	.0120	.0020
A39	.003	.003	.0030	.0000	.010	.008	.0090	.0010
A40	.004	.002	.0030	.0010	.004	.006	.0050	.0010
A41	.005	.007	.0060	.0010	.015	.011	.0130	.0020
A42	.012	.013	.0125	.0005	.038	.038	.0380	.0000
A43	.009	.010	.0095	.0005	.019	.021	.0200	.0010
A44	.011	.015	.0130	.0020	.034	.030	.0320	.0020
A45	.015	.017	.0160	.0010	.044	.048	.0460	.0020
A46	.007	.008	.0075	.0005	.018	.021	.0195	.0015
A47	.004	.004	.0040	.0000	.010	.010	.0100	.0000
A48	.007	.008	.0075	.0005	.019	.016	.0175	.0015
A49	.015	.010	.0125	.0025	.026	.026	.0260	.0000
A50	.006	.010	.0080	.0020	.032	.033	.0325	.0005
A57	.006	.007	.0065	.0005	.016	.020	.0180	.0020
A58	.004	.008	.0060	.0020	.014	.016	.0150	.0010
A59	.014	.009	.0115	.0025	.030	.026	.0280	.0020
A60	.009	.008	.0085	.0005	.021	.022	.0215	.0005
A61	.010	.009	.0095	.0005	.029	.032	.0305	.0015
A62	.007	.007	.0070	.0000	.018	.019	.0185	.0005
A63	.013	.012	.0125	.0005	.032	.036	.0340	.0020
A65	.010	.008	.0090	.0010	.025	.022	.0235	.0015
A66	.011	.011	.0110	.0000	.017	.019	.0180	.0010
A67	.011	.012	.0115	.0005	.031	.027	.0290	.0020
A81	.011	.009	.0100	.0010	.028	.025	.0265	.0015
A223	.014	.012	.0130	.0010	.029	.025	.0270	.0020

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[1] Duplicate lots A and B.

TABLE 2.—*Arsenic and lead (grain per pound) found in duplicate samples of apples*—Continued

Sample	Arsenic as As_2O_3				Lead			
	A	B	Mean	Deviation	A	B	Mean	Deviation
A224	0.012	0.010	0.0110	0.0010	0.020	0.019	0.0195	0.0005
A225	.008	.009	.0085	.0005	.019	.020	.0195	.0005
A226	.019	.014	.0165	.0025	.025	.015	.0200	.0050
A227	.018	.012	.0150	.0030	.025	.021	.0230	.0020
A228	.012	.017	.0145	.0025	.020	.022	.0210	.0010
A233	.007	.009	.0080	.0010	.022	.017	.0195	.0025
A261	.020	.020	.0200	.0000	.032	.029	.0305	.0015
A304	.009	.010	.0095	.0005	.022	.026	.0240	.0020
A305	.005	.004	.0045	.0005	.014	.014	.0140	.0000
A306	.009	.009	.0090	.0000	.016	.014	.0150	.0010
A307	.004	.003	.0035	.0005	.012	.012	.0120	.0000
A312	.004	.004	.0040	.0000	.012	.014	.0130	.0010
A313	.002	.004	.0030	.0010	.008	.010	.0090	.0010
A316	.006	.004	.0050	.0010	.012	.010	.0110	.0010
A317	.003	.004	.0035	.0005	.011	.010	.0105	.0005
A318	.040	.035	.0375	.0025	.100	.090	.0950	.0050
A319	.024	.026	.0250	.0010	.075	.078	.0765	.0015
A322	.003	.006	.0045	.0015	.008	.012	.0100	.0020
A323	.003	.003	.0030	.0000	.009	.007	.0080	.0010
Average	.00950	.00938	.00944	.00070	.02255	.02235	.02246	.00152

The ratio Pb/As_2O_3 in the mean values is 2.37/1, and the ratio average deviation Pb/average deviation As_2O_3 is 2.15/1. These ratios, as well as the percentage values given in the previous paragraph, indicate that the error of the lead determination is not so great as that of the arsenic determination. Apparently the error of sampling affects the accuracy of the determination of these elements to much the same degree, and the increased accuracy of the lead determination reflects the greater accuracy of the chemical part of the procedure, mentioned previously. It is to be noted that the mean values for both arsenic and lead are close to the amounts permitted by law, and therefore the estimations of accuracy should be particularly applicable to samples falling in this range.

SUMMARY AND CONCLUSIONS

The errors incident to the determination of lead and arsenic as spray residues on apples are due to two sources: Errors of sampling and errors in the chemical determination of the elements. The latter may be further divided into errors of technique and errors of standardization.

When the chemical determination of lead by the photoelectric method is considered separately, the probable error was ±0.0028 mg, when samples each containing 0.4000 mg of lead were analyzed. For the entire procedure, including both the chemical determination and the sampling, the mean amount of As_2O_3 present was 0.00944 grain per pound of fruit, with an average difference between duplicates of 0.00140 grain per pound. For lead the mean values were 0.02246 grain per pound of fruit, with an average difference between duplicates of 0.00304 grain per pound. These are the mean values of 164 apple samples analyzed in duplicate.

The lead determination was slightly more accurate than the Gutzeit arsenic estimation, even when the errors due to sampling were considered. The average deviation of individual arsenic determinations from the mean of duplicate determinations was 7.4 percent of the mean; for lead it was 6.8 percent of the mean. These data are based on the analysis of 164 samples.

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Vitamins in Theory and Practice. By Leslie J. Harris, Sc.D., D.Sc., University of Cambridge. The Macmillan Company, 60 Fifth Avenue, New York, N. Y., 1935. xix + 240 pp. Illustrated. 15.5 × 21 cm. Price, $3.00.

The author states that "this little book forms the subject matter of a series of four 'Thursday afternoon' lectures given at the Royal Institution, London, in 1934." The book is not written for biochemists and physiologists but, to use the author's own words, is designed to present "a readable narrative of that truly romantic subject, the history of vitamin discovery and research, which I hope may be intelligible and of interest to the general reader." That the author has succeeded in this regard, there can be no question.

While the book will appeal to all groups, it can be read with pleasure and profit by all chemists in spite of the fact that Professor Harris (who is a recognized authority in the field of vitamin research) has attempted to avoid a detailed discussion of vitamin chemistry. He has included sufficient chemical data, however, to give the general reader a good conception of the recent advances that have been made during the past few years. The topics are discussed under the following chapter headings: Chapter 1. The discovery of vitamins; Chapter 2. How many vitamins are there? Chapter 3. Beriberi and vitamin B_1; Chapter 4. Pellagra and vitamin B_2; Chapter 5. Scurvy and vitamin C; Chapter 6. Vitamin D and rickets; Chapter 7. Vitamin A; Chapter 8. Vitamin E—Diet and sterility; Chapter 9. Dietetics—What to eat.

In chapter 9 the author stresses the practical and clinical importance of vitamins in nutrition and makes some practical suggestions on "what to eat." This is followed by a brief summary entitled "Retrospect."

The book is written in a simple but interesting style and is replete with whimsical humor and philosophy. Professor Harris combines the rare qualities of the scientist and of the journalist to produce an accurate and up to date scientific narrative "that reads like a novel."

The reviewer recommends this book to all readers who desire a brief and authoritative discussion of vitamins in theory and practice.

R. Adams Dutcher

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THE EFFECTS OF BREED CHARACTERISTICS AND STAGES OF LACTATION ON THE VITAMIN C (ASCORBIC ACID) CONTENT OF COW'S MILK [1]

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ONE FIGURE

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While milk is not usually considered a vitamin C rich food, from the standpoint of infant feeding it does constitute an important source of this dietary essential. In fact Hess ('20) has reported that one pint of fresh raw milk per day furnishes sufficient vitamin C for the average infant. Chick, Hume and Skelton ('20) found, however, that about 85 ml. of milk were required to furnish a minimum protective dose of vitamin C for the guinea pig. Hart, Steenbock and Ellis ('20) have reported that 50 ml. of summer milk or 75 ml. of winter milk were required to furnish a minimum protective dose of vitamin C, while Dutcher, Eckles, Dahle, Mead and Schaefer ('20) reported that the quantity of milk required to furnish this amount of vitamin C depends upon the diet of the cow and was found to range from 20 to 60 ml.

Results of investigations other than those reported above emphasized the importance of the diet of the cow on the vitamin C content of the milk produced, but a review of the available literature failed to indicate that any systematic study had been made concerning the effect of breed characteristics or of stage of lactation on the vitamin C content of milks produced under comparable conditions.

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Since milk constitutes an important item in the human diet and since the importance of an adequate amount of vitamin C in the diet is becoming more fully appreciated, it appeared to us that any investigation that might yield further information as to how milks containing greater quantities of this dietary essential might be produced would be worth while.

The more recent investigations concerning the nature and the reactions of vitamin C (ascorbic acid) (King and Waugh, '32; Waugh and King, '32; Harris and Ray, '33; Herbert, Hirst, Percival, Reynolds and Smith, '33; Reichstein, Grussner and Oppenauer, '33; Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey '33; Bessey and King, '33) appeared to suggest a more direct technic that might be used in carrying out such an investigation. This, together with the fact that the college herd is composed of cows of the various standard breeds, representing different stages of lactation and receiving a uniform diet of definite composition, offered further inducement toward such a study. In fact many of the essentials for a comprehensive study of the effects of both breed characteristics and stages of lactation on the vitamin C content of cow's milk were available.

EXPERIMENTAL

Samples of freshly drawn milk (morning) from cows comprising the college herd were collected and taken directly to the laboratory where the determination of their ascorbic acid content was started at once. These cows were receiving a typical dairy ration which contained weighed quantities of green feed. The ascorbic acid content of the various samples of milk was determined in the following manner: 25 ml. of the fresh milk were treated with 25 ml. of a 16 per cent trichloracetic acid solution in order to precipitate the proteins. The supernatant liquid was removed from the precipitated materials by centrifuging and decanting. The precipitated residue was then washed successively with 20 and 10 ml. portions of an 8 per cent trichloracetic acid solution. These washings were combined with the supernatant liquid and the total volume titrated with a standardized solution of 2–6 dichlorophenolindophenol. This 2–6 dichlorophenolindophenol was prepared by dissolving 0.1 gm. of the dye in successive portions of warm distilled water, filtering and diluting to 200 ml. The filtered dye solution was then standardized against a N/100 iodine solution and also against pure ascorbic acid before being used. A fresh dye solution was prepared for each day's titration.

DATA

Since space does not permit the presentation of data obtained from each individual cow used in the studies pertaining to the effects of breed characteristics on the ascorbic acid content of the milk, only the data from cows selected as representative of each of the five respective breeds are presented in table 1. In selecting these representative cows, due consideration was given to such factors as stage of lactation, age of cow, milk production, etc. In addition, all cows which showed any detectible evidence of having mastitis were eliminated from consideration. When considered, therefore, from the standpoint of these factors, the cows comprising the several groups were as nearly representative of the respective breeds as was possible to select from the number comprising the college herd.

In the studies relative to the effect of the stage of lactation on the ascorbic acid content of milk, data from all cows were considered in the tabulation. That, is, all cows regardless of breed, age, milk production, etc., were tabulated in monthly groups in accordance with the stages of lactation. The average ascorbic acid contents of the milks for the various stages of lactation were then calculated in terms of milligrams per quart and are presented graphically in figure 1.

DISCUSSION

In the studies relative to the influence of breed characteristics on the ascorbic acid content of cow's milk, the milks

TABLE 1

Giving the ascorbic acid content of a series of milk samples produced by representative cows from five dairy breeds

BREED	NUMBER OF COW	APPROXIMATE AGE OF COW AT START OF EXPERIMENT	ASCORBIC ACID CONTENT OF MILK PER QUART						
			Sample taken 8/1/34	Sample taken 8/4/34	Sample taken 8/11/34	Sample taken 8/20/34	Sample taken 9/1/34	Sample taken 9/8/34	Average
		months	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Ayrshire	1392	92	10.4	12.1	10.5	11.1	12.6	12.2	11.5
Ayrshire	1439	81	14.7	14.6	13.9	17.0	14.2	15.3	14.9
Ayrshire	1477	77	14.3	10.6	12.2	12.5	12.7	12.0	12.4
Ayrshire	1754	39	14.9	12.6	10.6	14.8	14.7	12.2	13.3
Ayrshire	1758	39	8.4	10.0	10.8	13.3	13.3	12.2	11.3
Ayrshire	1770	37	15.9	12.9	13.9	14.2	13.9	12.5	13.9
Average for the breed		61	13.1	12.1	12.0	13.8	13.6	12.7	12.9
Brown Swiss	1551	72	14.6	15.2	17.7	15.2	15.3	12.2	15.0
Brown Swiss	1559	70	11.6	11.6	9.7	10.5	16.3	13.8	12.2
Brown Swiss	1611	64	13.3	14.8	12.2	12.5	13.5	13.4	13.3
Brown Swiss	1615	64	17.6	19.8	19.2	22.0	20.0	18.3	19.5
Brown Swiss	1619	67	12.5	11.5	10.5	12.0	14.6	12.0	12.2
Brown Swiss	1734	42	15.9	15.0	15.7	16.9	16.8	18.0	16.4
Average for the breed		63	14.3	14.6	14.2	14.9	16.1	14.6	14.8
Guernsey	1484	118	12.7	12.6	14.6	16.0	17.1	14.2	14.5
Guernsey	1545	79	12.6	11.5	11.1	13.1	14.7	12.4	12.6
Guernsey	1548	76	11.9	10.5	11.8	13.1	13.5	11.7	12.1
Guernsey	1767	40	14.6	14.9	11.2	11.4	12.5	12.4	12.8
Guernsey	1771	37	18.5	14.3	14.3	16.5	16.3	17.1	16.2
Guernsey	1797	34	12.5	11.4	8.9	7.0	9.9	8.4	9.7
Average for the breed		64	13.8	12.5	12.0	12.8	14.0	12.7	13.0
Holstein	1440	83	9.4	10.7	9.4	9.8	9.8	10.0	9.9
Holstein	1646	58	9.8	10.1	10.0	11.2	11.3	11.7	10.7
Holstein	1656	56	9.9	8.3	10.1	8.5	10.3	10.0	9.5
Holstein	1698	52	11.7	9.5	8.6	8.6	11.3	12.7	10.4
Holstein	1744	41	11.0	10.2	11.3	12.5	10.5	10.1	10.9
Holstein	1805	32	9.2	7.0	7.3	10.8	8.4	7.8	8.4
Average for the breed		54	10.2	9.3	9.4	10.2	10.3	10.4	10.0
Jersey	1480	74	15.2	15.4	14.2	15.3	15.2	15.9	15.2
Jersey	1501	77	10.1	11.4	10.4	12.9	12.8	10.1	11.3
Jersey	1703	112	15.4	14.2	11.3	14.6	13.5	13.9	13.8
Jersey	1717	69	15.8	14.6	14.6	15.1	17.1	19.3	16.1
Jersey	1793	53	13.1	12.5	10.0	8.4	14.1	12.1	11.7
Jersey	1821	46	12.1	13.5	12.4	7.4	12.1	11.8	11.6
Average for the breed		72	13.6	13.6	12.2	12.3	14.1	13.8	13.3

from a total of seventy-nine cows were collected. This group of cows was composed of: 14 Ayrshires, 13 Brown Swiss, 9 Guernseys, 27 Holsteins and 16 Jerseys. A few of these cows were known to have mastitis, but when it was found that mastitic milks gave abnormal ascorbic acid values such cows were eliminated from consideration. Milks from sixty-one cows were titrated at each of the six titration periods, while milks from eleven other cows were titrated from two to five times during the 39-day experimental period. These cows ranged from 3 to 10 years of age and represented all stages of lactation.

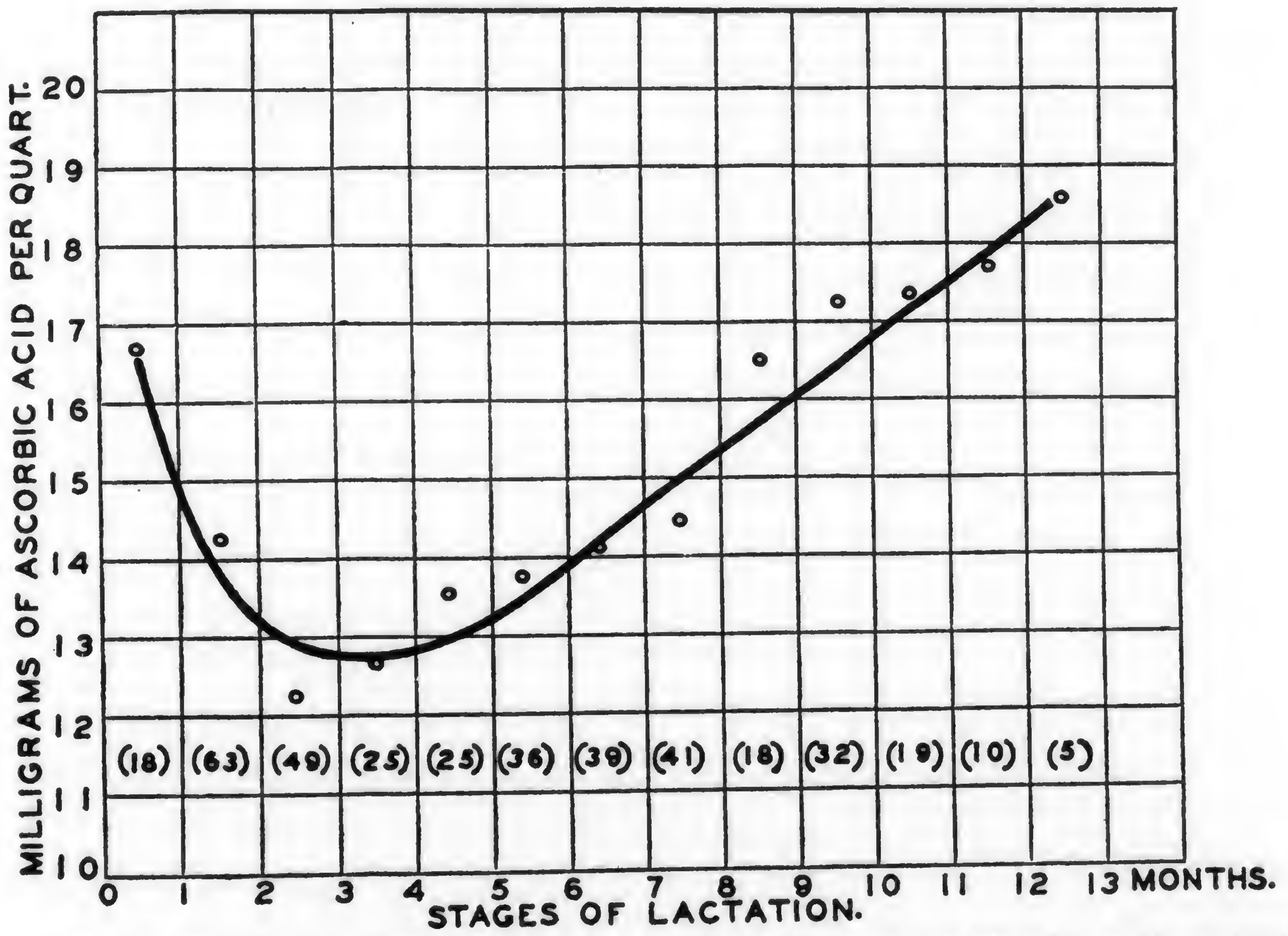

Fig. 1 Showing the effect of the stage of lactation on the ascorbic acid content of the milk produced. The numbers given in enclosure represent the number of samples of milk titrated during the respective periods.

It may be observed from the data given in table 1 that the ascorbic acid content of the various milk samples varied with the cow almost as much as it varied with the breed. It may also be observed from the data given in this table that the ascorbic acid content of milk from some cows showed slight variations from period to period. While no explanation is offered for the latter variation, it seems highly probable that at least part of the variation in the ascorbic acid content of milks produced by cows of the same breed is due to different stages of lactation.

The data presented, while not showing sharp differences in the ascorbic acid content of milks from cows of different breeds, indicate that slight differences do exist in the case of certain breeds. While the average ascorbic acid content of milks from cows of the Ayrshire, Guernsey and Jersey breeds was found to be approximately the same, the average ascorbic acid value of thirty-six samples of milk from Brown Swiss cows was 48 per cent above that obtained for a similar number of milk samples from Holstein cows. This difference in ascorbic acid content could not be explained on the basis of the difference in the volume of milk produced, as the average volume of milk produced by the two breeds during the experimental periods was found to be approximately equal.

In the studies relative to the effect of the stage of lactation on the ascorbic acid content of cow's milk, a total of 410 samples of milk were considered. These samples of milk represented all stages of lactation from post-parturition to the thirteenth month of milk production. As was the case in the studies relating to the effects of breed characteristics, these samples of milk were collected directly from the respective cows and the ascorbic acid content determined at once by titration with a standard solution of the 2–6 dichlorophenolindophenol. In order to conserve space, the data obtained in this phase of the investigation have been arranged in accordance with the stage of lactation of the respective cows and are presented in graphic form.

It will be observed from these data (fig. 1) that the average ascorbic acid content of milks produced by a mixed dairy herd, receiving similar rations, varied widely according to stages of lactation. Eighteen samples of such milk, produced during the first month of lactation, were found to contain an average of 16.7 mg. of ascorbic acid per quart, while forty-nine samples of milk produced during the third month of lactation contained an average of only 12.3 mg. per quart, and five samples of milk produced during the thirteenth month of lactation contained 18.6 mg. per quart. Samples of milk taken at intermediate stages of lactation gave intermediate ascorbic acid values.

A few samples of colostrum were also titrated and in each case the ascorbic acid content was found to be unusually high. This high ascorbic acid content of colostrum was believed to indicate a certain degree of storage of this substance by the cow during the pre-parturition period. The data presented in figure 1 show, however, that if such a body-store of ascorbic acid does exist it must be limited, since 2 months after parturition the amount of this substance in the milk has dropped to a minimum. After the first 2 months of lactation, the ascorbic acid content of milk is apparently dependent solely upon the ascorbic acid content of the diet of the cow. With a constant intake of ascorbic acid in the diet of the cow and a decrease in milk production such as usually occurs in the later stages of lactation, one might expect milk of higher ascorbic acid content, especially if the storage capacity of the cow for this substance is limited.

A point which is probably worthy of mention in this connection concerns the apparent relation between 'cardboard-flavor' and low ascorbic acid content of milks. It was observed, invariably, that those samples of milk, portions of which had been titrated for ascorbic acid content, which developed cardboard-flavor on standing, decreased in ascorbic acid content coincident with the formation of the off-flavor. Whether cardboard-flavor is due to the degradation products of ascorbic acid or whether those factors instrumental in the formation of cardboard-flavor are also instrumental in the destruction of ascorbic acid was not determined.

SUMMARY

A study has been made concerning the effects of breed characteristics and stages of lactation on the ascorbic acid content of cow's milk. As a result of these studies, the following conclusions are drawn:

1. Cows of the same breed, while receiving similar diets, may produce milks which show wide variations in their ascorbic acid content. Such variations in ascorbic acid content are thought to be due, in part at least, to differences in stages of lactation.

2. Cows of different breeds, while receiving similar diets, produced milks which differed somewhat in their average ascorbic acid values. Of the five breeds studied, the Brown Swiss cows produced milks of the highest ascorbic acid content, while the Holstein cows produced milks of the lowest ascorbic acid value.

3. Stage of lactation appeared to have a more definite effect upon the ascorbic acid content of milk than did breed differences. The ascorbic acid content of milk was found to be relatively high during the early stages of lactation, but decreased to a minimum after about 2 months of lactation, and then increased to a maximum in the later stages of lactation.

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CIRCULAR 175 JUNE, 1936

FEEDING GROWING TURKEYS

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
DIVISION OF AGRICULTURAL EXTENSION
STATE COLLEGE, PENNSYLVANIA

DIGEST

From a series of feeding tests it was found that a turkey starter of 24 per cent crude protein (figured on dry feed basis) is a satisfactory protein level for starting poults.

At eight weeks of age the protein requirement is less and it continues to decrease as the turkeys approach maturity.

Vitamin A is an essential growth vitamin and it is supplied through yellow corn, alfalfa, greens, and fish oils.

Growing turkeys require about double the amount of vitamin D as growing chickens. One pint of a good grade of natural oil should supply enough vitamin D for each 100 pounds of feed consumed.

The vitamin G requirement is relatively high in the early stages of growth. It is supplied by milk or milk products.

Fish meal or fish oil may produce a fishy odor in the meat, and to be certain of avoiding this trouble fish products should be discontinued from the ration 6 to 10 weeks before marketing time.

Carbohydrates, fats, and minerals are supplied in ample and safe amounts in the mash formulae given in this circular.

Vegetable proteins, like corn gluten meal and soybean oil meal, can be partially substituted for animal proteins.

Baby poults may be slow to learn to eat. For this reason feed should be placed before them as soon as possible.

Coarse feeds, such as oats, barley, and legume hay, help to control feather picking.

Green grass range will help to reduce the grain and mash consumption.

When liquid milk is fed, the drinking utensils should be cleaned frequently.

FEEDING GROWING TURKEYS

J. E. HUNTER AND H. H. KAUFFMAN

Protein Requirements

IN 1929 The Pennsylvania State College published in Bulletin 250 evidence showing that growing turkeys require a ration containing more protein than that required by growing chickens. In this investigation the growing turkeys were supplied with a chick starting mash containing 16½ per cent protein and liquid milk was kept before them at all times. Careful records of mash and milk consumption were kept. By calculations made from the consumption records of mash and milk it was found that the protein consumption was quite high for the early growing period and gradually decreased as the birds became older.

As an outgrowth of this work a turkey starting mash containing 24 per cent crude protein is recommended to be fed in all mash form for eight weeks. A developer mash containing 20 per cent is substituted for the starting mash after eight weeks of age. The feeding of grain is started at 12 weeks of age and kept before the birds at all times. This feeding of unlimited quantities of scratch grain gives the birds an opportunity to lower their protein intake as they grow older by increasing the consumption of grain.

Vitamin Requirements

Growing turkeys should be supplied with ample quantities of vitamin A from yellow corn, alfalfa or other green feed. Some fish oils supplied primarily as a source of vitamin D also contain considerable amounts of vitamin A.

Turkey poults when deprived of access to direct sunlight are particularly susceptible to rickets if ample quantities of vitamin D are not added to the ration. The common sources of vitamin D added to turkey mashes are cod liver oil or other vitamin D fish oils. Research work has shown that young growing turkeys require double the amounts of vitamin D required by growing chickens. To amply protect young turkeys against rickets if they are confined without access to sunlight, one pound (one pint) of a good grade of vitamin D oil should be added to each 100 pounds of mash if the ration is to be fed in all mash form. If grain is fed in the early growing period, additional oil should be supplied either in the mash or on the grain. Since the vitamin D requirements of growing turkeys are high, it is a good practice to feed some oil even though the birds have access to sunshine, especially with late hatched poults.

Young growing turkeys require considerable amounts of vitamin G for best growth and development. Milk or milk products are rich in this factor and should be used liberally in rations for young turkeys. As the poults grow older their requirements for vitamin G decrease.

Danger of a Fishy Flavor and Odor in Turkey Meat

It has been observed by a number of investigators that turkey meat may have a definite fishy taste or odor at marketing time.. It is generally believed that this is due to either the oil fed to supply vitamin D or to fish meal in the mash. If the birds are on range or confined with access to direct sunlight, it is a safe procedure to eliminate the vitamin D supplement at eight weeks when the starting mash is discontinued. If the birds are confined without access to sunlight, some vitamin D oil should be supplied after eight weeks. This should be discontinued for at least four weeks before marketing time. Many growers to be doubly sure of eliminating the fishy flavor or odor from their market birds remove both the fish oil and fish meal from the mash 6 to 10 weeks prior to marketing time.

Mineral Requirements of Turkeys

Little information is available in regard to the mineral requirements of growing turkeys. Some data have been presented, however, to show that the requirements of the growing turkey for calcium and phosphorus are higher than those of growing chickens. Turkeys are extremely susceptible to hock disease, sometimes called slipped tendons or perosis, an abnormality aggravated by excessive quantities of calcium and phosphorus. Since animal protein products are generally used liberally in turkey rations and since these materials contain considerable quantities of calcium and phosphorus, turkey mashes in general contain more calcium and phosphorus than do chick mashes. It is a safe practice generally to assume that ample quantities of phosphorus are supplied by the ingredients making up a high protein turkey mash. These mashes usually do not contain excessive amounts of calcium and it is the common practice to supply the birds with some additional form of calcium such as oyster shell or limestone grit fed in separate hoppers.

Nutrients

In addition to proteins, vitamins, and minerals, turkeys require carbohydrates, fats, and water. The feeding of scientifically compounded mashes will supply adequate amounts of carbohydrates and fats required by the birds. Since water plays an important role in growth, the necessity of an ample supply of clean fresh water cannot be over-emphasized.

Use of Vegetable Protein Concentrates

The value of high protein turkey mashes has been demonstrated for several years. The cost of a high protein mash is necessarily greater per ton than the cost of a mash of similar quality ingredients but containing a lower level of protein. An investigation to determine whether or not lower priced vegetable protein concentrates might be used to replace some of the higher priced animal protein products was inaugurated. It was not the purpose of this investigation to replace all of the animal protein but only a part of it as a variety of protein carriers is desirable in formulating a ration for maximum results. From this investigation it was apparent that considerable quantities of vegetable protein in the form of soybean oil meal or corn gluten meal can be used to replace a portion of the dried milk, meat meal, or fish meal. There are other vegetable protein products that might be used in this connection but only soybean oil meal and corn gluten meal have been studied. These concentrates are generally available in quantity and ordinarily sell for a lower price on a basis of their protein content than do milk, meat meal, or fish meal. The vegetable protein concentrates do not have the mineral content that the animal protein concentrates carry and for this reason when they are used to replace animal protein, mineral adjustments are necessary.

Corn Gluten Meal Promotes Early Fleshing

Soybean oil meal and corn gluten meal functioned with equal efficiency from the standpoint of growth. The birds receiving corn gluten meal, however, showed a heavier fleshing condition at an early age as characterized by a covering of flesh over the breast. This particular source of protein should be valuable in meeting the requirements of producers attempting to finish turkey broilers or those interested in marketing a portion of their crop prior to the Thanksgiving season. It also offers possibilities for finishing late hatched poults in order to market them during the Christmas season when there is a demand for small, well-finished turkeys.

The following rations have been compounded to contain ingredients that can be raised on Pennsylvania farms or purchased on Pennsylvania markets. They also supply all the nutrients in balanced proportions that are needed for fast, economical growth and good finish. The first column gives the formula for a 24 per cent protein starter using only animal concentrates as the principal source of the protein supply.

Rations

	24% Protein Turkey Starters		*20% Protein Turkey Developers*	
	Using animal protein concentrates	*Using animal and vegetable protein concentrates*	*Using animal protein concentrates*	*Using animal and vegetable protein concentrates*
Yellow corn meal	280	260	310	270
Wheat bran	100	100	150	150
Wheat middlings	100	100	150	150
Ground heavy oats	100	100	100	100
Alfalfa meal	50	50	50	50
Dried milk	100	50	50	40
Meat scraps	150	150	170	70
Fish meal	100	50	..	..
Salt	10	10	10	10
Cod liver oil	10	10	..	..
Soybean oil meal or corn gluten meal *	..	100	..	140
Calcium carbonate	..	20	10	20
	1000	1000	1000	1000

* Soybean oil meal, corn gluten meal, or a blend of the two may be used interchangeably.

The second column gives the formula for a ration in which part of the animal concentrates are replaced by corn gluten meal or soybean oil meal. This is done to produce a ration that will cost less when the price of the animal concentrates are relatively higher in price. The developer formulae are given in the third and fourth columns respectively.

Growth and Feed Consumption Records for Bronze Turkeys*

	Body Weight Pounds Per Bird		Total Feed Pounds Per Bird Per Period	Total Feed Pounds Per Bird To Date
	Males	*Females*		
Day old	.11	.12	..	..
0— 4 weeks	.72	.63	1.50	1.50
4— 8 weeks	2.41	1.97	4.00	5.50
8—12 weeks	5.80	4.76	9.00	14.50
12—16 weeks	10.06	7.69	14.50	29.00
16—20 weeks	15.02	10.64	17.50	46.50
20—24 weeks	19.01	12.46	18.50	65.00

* Pennsylvania State College Bulletin No. 250.

These birds were raised in confinement during the entire growing period and did not receive succulent green feed. The 24 per cent starter and 20 per cent developer with the animal protein concentrates were fed.

The Relation of Feed Consumption and Growth*

Age in Weeks	*Feed to Produce One Pound of Gain* Pounds
1— 5	2.22
5— 9	2.74
9—13	3.55
13—17	5.70
17—21	7.08
21—25	10.46
Ave. 1—25 weeks	5.42

* Pennsylvania State College Bulletin No. 321.

Feeding Suggestions

Baby poults are slower than chicks to establish the habit of eating. For this reason feed should be placed before them at an early age. Should they not eat before 60 hours of age, some may become weakened and die from lack of feed. Placing hoppers in a light place in the brooder house makes it easier for them to find the feed. Placing an electric light just above the feed hoppers is a help, especially if the brooder house is rather dark.

A few turkey raisers have adopted the practice of placing a few older poults in with each group of those just hatched. The older ones teach the younger ones how to eat. Of course these should be removed as soon as the younger ones have learned to eat. Giving them liquid milk at the start is a means of getting some feed in their bodies at an early age. This practice should not be continued if a high protein turkey starter is fed.

Provide Plenty of Hoppers

A sufficient number of hoppers should be provided to permit at least one half of the poults to eat at one time. At the start they prefer to eat from an open type of container, because they do not have to push their heads through bars or other obstructions. When metal hoppers with oval feeding spaces are used, they should be replaced as soon as the young turkeys strike the upper part of their beaks on the metal. Deformed beaks may result from injuries on hoppers.

Turkeys form the habit of wiping their beaks on the feathers of other turkeys. Wires stretched across the hoppers to provide a means of cleaning beaks may lessen the trouble from feather picking. It is common for turkeys to start feather picking in the fall when they can obtain very little roughage. Feeding heavy whole oats, barley, and legume hay will help to satisfy this craving.

Feed Containers Should Aid Sanitation

The overfilling of hoppers and the use of an unsatisfactory type results in a tremendous waste of feed. Not only should one consider the waste but also the unsanitary practice of allowing poults to pick up feed from the floor which is likely to be contaminated. Placing all hoppers on low wire frames will prevent poults from eating feed which has spilled from the hoppers and a similar practice for water fountains will keep poults away from damp areas. Hoppers and fountains on range should be moved frequently to clean areas.

Green Feed and Pasture

Greens are not essential with the recommended rations but there are turkey growers who prefer to feed some. If long stringy greens like grass and alfalfa are fed in the houses, they must be cut fine. Poults may swallow whole stalks, which can cause a stoppage in the digestive tract. This trouble seldom occurs when they pasture from standing stalks.

Good pastures help to cut down the grain and mash consumption after turkeys are placed on range. The grasses and legumes that are used in Pennsylvania for pastures are satisfactory for turkeys. Rape is used and can produce much good pasture per acre. It is very responsive to fertile soils, rains, and good growing weather and may be ready to pasture six weeks after seeding. A rape pasture may furnish feed for 90 days. For late forage it should be seeded from the first to the middle of July, but rape may be sown as early as oats planting time. If broadcast, it is seeded at the rate of eight pounds per acre and harrowed shallowly, or five pounds per acre when drilled. An acre will furnish forage for 75 to 125 turkeys.

Grain and Water Consumption

Water is necessary at all times and should be kept continually before the turkeys. If enough liquid milk is available to supply half the drink, the dried milk can be eliminated from the mash. After the turkeys are 12 weeks old, a smaller proportion of liquid milk will meet the requirements. Milk utensils should be scalded or washed with a disinfectant at least every few days.

Turkeys eat more grain in proportion to mash when high protein mashes are fed than if mashes of low protein content are used. Cold weather also stimulates an appetite for grain. For this reason, late hatched poults usually start heavier grain consumption at a younger age than early hatched birds. As turkeys approach maturity, the grain consumption usually increases and the mash consumption decreases. A flock of May hatched birds may consume one part of grain to four or five of mash in September. By December the feed consumption may be four parts of grain to one of mash.

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BULLETIN 331 JUNE, 1936

The Absorption of Plant Nutrients in Relation to the Production of Pennsylvania Cigar-Leaf Tobacco

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
STATE COLLEGE PENNSYLVANIA
In cooperation with the
BUREAU OF PLANT INDUSTRY
UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

The Absorption of Plant Nutrients in Relation to the Production of Pennsylvania Cigar-Leaf Tobacco[1] [2]

J. J. THOMAS AND D. E. HALEY, THE PENNSYLVANIA STATE COLLEGE, AND
OTTO OLSON, BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT
OF AGRICULTURE

Introduction

INVESTIGATIONAL work on Pennsylvania cigar-leaf tobacco was initiated by The Pennsylvania State College in 1893. Later, in 1912, with the cooperation of the Bureau of Plant Industry, United States Department of Agriculture, tobacco substations were established at Lock Haven, Clinton county, and at Ephrata, Lancaster county. In 1931 the Ephrata station was abandoned and the various field experiments relating to tobacco have since been conducted at a new substation situated on the Lefever farm near Roseville, just one mile north of the city of Lancaster. Field experiments, however, are conducted as before at the Lock Haven substation.

Lancaster county is the center of production of cigar-leaf tobacco in Pennsylvania and it is for this reason that the major part of the investigations relating to cigar-leaf tobacco has largely been confined to this area.

Considerable attention is now being given at the Roseville substation to those factors which have to do with yield and quality, but other important phases of tobacco production are likewise given due consideration.

The Experimental Field

The experimental field at Roseville comprises 10 acres in area and is practically level. Although not limed since 1910, the soils are quite alkaline; having a reaction approximately equal to a pH of 8.0. The field was allowed to remain uncropped for the three seasons preceding 1932, and as no fertilizer had been applied for a long period previous to 1932, the soils were in a low state of productivity at that time.

The Soil

In 1931 a detailed soil survey of this experimental field was made by Dr. A. L. Patrick of The Pennsylvania State College, who found that two types of soil were represented, the Hagerstown silt loam and the Hagerstown clay loam. These types are indicated in Fig. 1.

The Hagerstown silt loam is typical of the Lancaster county area. The brown surface soil, the A-Horizon, extends to a depth of 8 to 10 inches and there is no change in texture until a depth of 13 to 15 inches is reached. Below this point there is a gradation into a pale yellow silt loam uncolored by organic matter.

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[1] Authorized for publication May 16, 1936. This investigation was conducted jointly by the Departments of Agricultural Biological Chemistry and Agronomy of the Pennsylvania Agricultural Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] Submitted by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agricultural and Biological Chemistry in the Graduate School of The Pennsylvania State College, 1935.

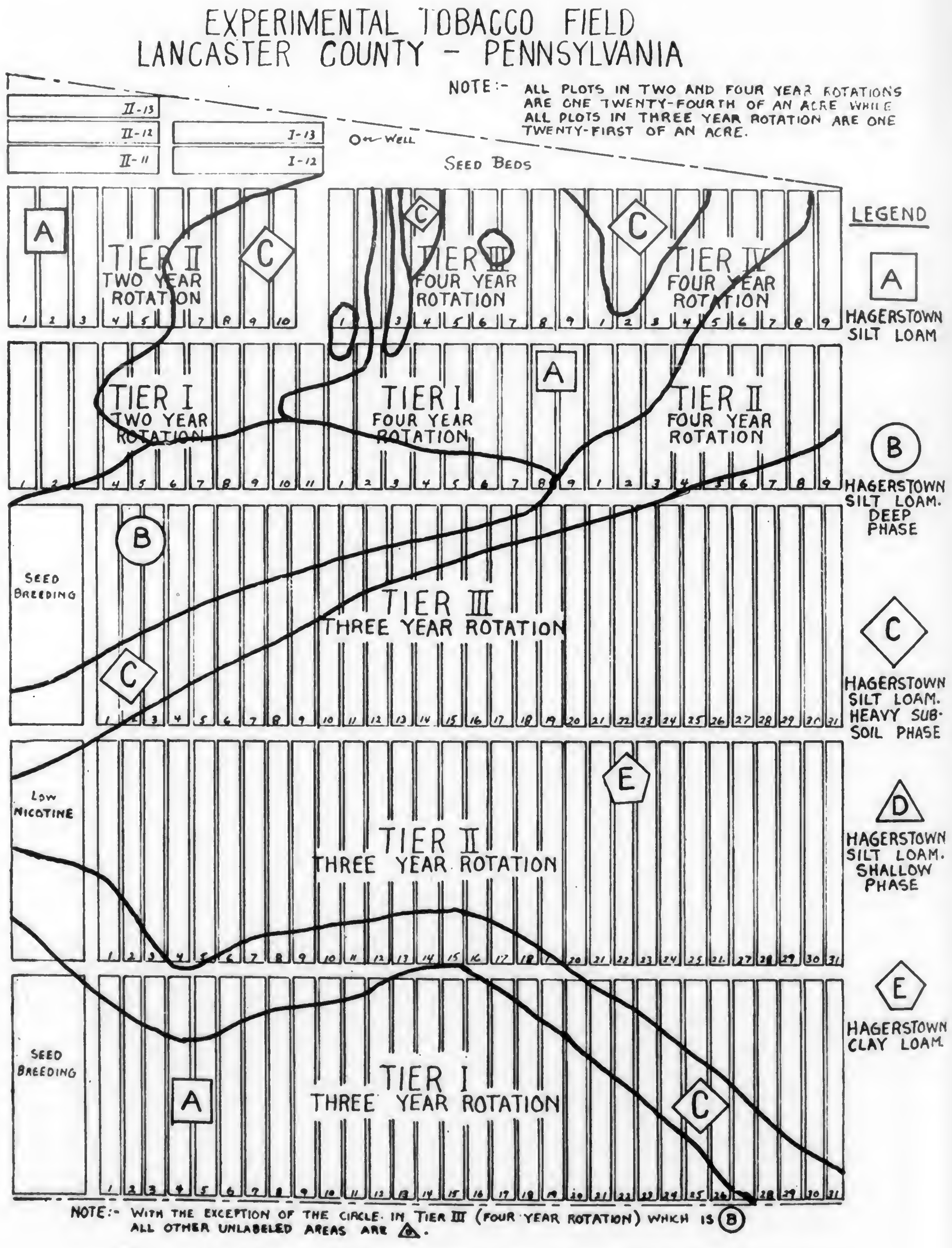
EXPERIMENTAL TOBACCO FIELD
LANCASTER COUNTY - PENNSYLVANIA
NOTE:- ALL PLOTS IN TWO AND FOUR YEAR ROTATIONS ARE ONE TWENTY-FOURTH OF AN ACRE WHILE ALL PLOTS IN THREE YEAR ROTATION ARE ONE TWENTY-FIRST OF AN ACRE.
II-13
II-12
II-11
I-13
I-12
OIL WELL
SEED BEDS
TIER II TWO YEAR ROTATION
TIER III FOUR YEAR ROTATION
TIER IV FOUR YEAR ROTATION
TIER I TWO YEAR ROTATION
TIER I FOUR YEAR ROTATION
TIER II FOUR YEAR ROTATION
SEED BREEDING
TIER III THREE YEAR ROTATION
LOW NICOTINE
TIER II THREE YEAR ROTATION
SEED BREEDING
TIER I THREE YEAR ROTATION
A
B
C
E
LEGEND
A HAGERSTOWN SILT LOAM
B HAGERSTOWN SILT LOAM. DEEP PHASE
C HAGERSTOWN SILT LOAM. HEAVY SUB-SOIL PHASE
D HAGERSTOWN SILT LOAM. SHALLOW PHASE
E HAGERSTOWN CLAY LOAM
NOTE:- WITH THE EXCEPTION OF THE CIRCLE IN TIER III (FOUR YEAR ROTATION) WHICH IS B ALL OTHER UNLABELED AREAS ARE D.

FIG. 1.—THE ROSEVILLE EXPERIMENTAL FIELD, SHOWING THE VARIOUS SOIL DIVISIONS AND THE PLOT ARRANGEMENTS

The B-Horizon is a reddish yellow friable clay loam which becomes heavier and more reddish in color to increasing depths and eventually grades into a brick red granular heavy clay. Borings were made to a depth of 40 inches but no bedrock was located.

The Hagerstown clay loam occupies the higher area in the central part of the experimental field. It is characterized by a reddish brown clay loam soil which grades at a depth of approximately nine inches into a granular heavy red clay subsoil.

The Rotation

As indicated in Fig. 1, that part of the experimental field devoted to a 3-year rotation system is divided into 3 tiers of 31 plots each, so that every year the three crops concerned, tobacco, wheat, and legumes, are represented—wheat following tobacco and legumes following wheat. Clover is the legume grown on all plots of the individual tiers with the exceptions of plots 29, 30, and 31 where it is replaced by alfalfa.

Each plot of the individual tiers represents 1/21 of an acre in area.

Field and Plot Treatments

When wheat was grown in the rotation a uniform treatment of superphosphate (16% P_2O_5) at the rate of 300 pounds per acre was given to the entire tier. The legumes received no supplemental additions. In the case of tobacco each plot received an individual treatment as indicated in Table 1. Manure was applied approximately one month before the tobacco was transplanted; the other fertilizers, with the exception of nitrate of soda which was applied as a side-dressing about 30 days after transplanting, were broadcast about one week before transplanting. However, plots 16, 17, and 18 were fertilized in the row at the time of transplanting.

Plot 13 received an application of hydrated lime at the rate of 1,000 pounds per acre and was the only plot receiving lime.

Sulfate of potash was the form of potash used, while superphosphate (16% P_2O_5) served as a carrier of phosphoric acid in every case.

On all plots except 14, cottonseed meal furnished two-thirds of the nitrogen applied, while nitrate of soda furnished the remaining one-third. Plot 14 received one-third of its nitrogen from cotton-seed meal and two-thirds from nitrate of soda.

Tier III on which tobacco was grown in 1934 was in legumes in 1933 and had lain fallow in 1932.

Object of These Investigations

The object of these investigations was to study the absorption of nitrogen, phosphorus, and potassium by tobacco plants during their growing season as modified by field treatment and other environmental conditions and, further, to seek a possible correlation between the total intake of these three important elements to the total yield and quality of the crop as measured by the production of "wrappers," "fillers," and "seconds."

Methods of Procedure

Four rows of tobacco, 66 plants to the row, were grown on each plot of Tier III of the 3-year rotation experiment at the Roseville substation in 1934.

TABLE 1.—THE FIELD TREATMENTS OF THE EXPERIMENTAL PLOTS, TIER III, OF THE 3-YEAR ROTATION SYSTEM. ALL QUANTITIES GIVEN ARE ON THE BASIS OF ONE ACRE.

Plot numbers	Manure	Fertilizer	Formula	Lime	Fertilizer applied	Reference
	tons	lbs.		lbs.		
1	----	1,000	6-8-4	----	Broadcast	N P 1/3 K
2	----	1,000	6-8-8	----	"	N P 2/3 K
3	----	1,000	6-8-12	----	"	N P K
4	----	1,000	6-8-16	----	"	N P 4/3 K
5‡	----	1,000	0-8-12	----	"	P K
6	----	1,000	3-8-12	----	"	1/2 N P K
7	----	1,000	9-8-12	----	"	3/2 N P K
8	----	1,000	6-0-12	----	"	N K
9	----	1,000	6-4-12	----	"	N 1/2 P K
10‡	----	1,000	0-8-12	----	"	P K
11	----	500	6-8-12	----	"	1/2 N 1/2 P 1/2 K
12	----	1,500	6-8-12	----	"	3/2 N 3/2 P 3/2 K
13	----	1,000	6-8-12	1,000	"	N P K L
14*	----	1,000	6-8-12	----	"	N P K
15‡	----	1,000	0-8-12	----	"	P K
16	----	500	6-8-12	----	In Row	1/2 N 1/2 P 1/2 K-R†
17	----	1,000	6-8-12	----	" "	N P K-R
18	----	1,500	6-8-12	----	" "	3/2 N 3/2 P 3/2 K-R
19	----	------	------	----	--------------	Check
20‡	----	1,000	0-8-12	----	Broadcast	P K
21	10	1,000	0-8-12	----	"	P K M
22	10	1,000	3-8-12	----	"	1/2 N P K M
23	10	1,000	3-8-8	----	"	1/2 N P 2/3 K M
24	10	500	3-8-12	----	"	1/4 N 1/2 P 1/2 K M
25‡	----	1,000	0-8-12	----	"	P
26	10	1,000	3-8-16	----	"	1/2 N P 4/3 K M
27	10	------	------	----	--------------	M
28	20	------	------	----	--------------	2 M
29§	----	------	------	----	--------------	Check
30‡ §	----	1,000	0-8-12	----	Broadcast	P K
31§	----	1,000	6-8-12	----	"	N P K

Reference Key: N = 60 lbs. nitrogen (N) /acre
P = 80 lbs. phosphoric acid (P_2O_5) /acre
K = 120 lbs. potash (K_2O) /acre
L = 1,000 lbs. lime ($Ca(OH)_2$) /acre
M = 10 tons manure/acre

* Two-thirds of nitrogen as nitrate of soda.

† Fertilizer broadcast unless indicated by symbol "R" which designates row application.

‡ Replicates.

§ Alfalfa in rotation instead of clover

During this particular growing season the sixth leaf from the bottom was taken from 11 representative plants of the middle two rows at each sampling. Therefore, in no case was more than one leaf sample taken from an individual plant.

The first sampling was made 46 days after the transplanting date while the following samples were made at approximately 10-day intervals until the date of harvesting. The samples were weighed immediately, packed in dry ice and brought to The Pennsylvania State College where they were dried at a temperature of 75°C. The dried leaves (lamina and midrib) were then ground and transferred to sample bottles and kept for analysis.

TABLE 2.—AVERAGE WEIGHT OF INDIVIDUAL LEAVES OF THE 1934 TOBACCO CROP AS BASED ON SAMPLES TAKEN FROM THE EXPERIMENTAL PLOTS OF TIER III AT DIFFERENT INTERVALS DURING THE GROWING SEASON. THE RESULTS ARE EXPRESSED ON MOISTURE-FREE BASIS.

Plot No.	Sampling			
	First	Second	Third	Fourth
	gm. per leaf	gm. per leaf	gm. per leaf	gm. per leaf
1	5.56	7.20	9.81	12.27
2	5.27	6.02	9.91	14.06
3	4.65	6.69	11.82	11.91
4	4.82	6.20	9.59	12.92
5	3.60	5.00	8.51	11.60
6	4.28	4.97	8.34	12.29
7	4.58	6.18	8.53	11.42
8	4.27	5.76	6.80	10.11
9	5.43	5.87	8.02	9.66
10	4.37	5.83	8.86	10.82
11	3.60	5.26	8.37	12.07
12	5.45	6.94	8.41	11.79
13	4.40	6.75	9.77	13.29
14	4.05	6.92	8.90	12.17
15	4.29	6.83	7.58	10.64
16	4.17	6.62	7.18	12.05
17	3.67	7.02	8.89	12.73
18	5.13	5.93	11.33	14.01
19	3.38	5.31	9.01	11.75
20	4.21	6.98	9.40	11.90
21	3.68	5.50	8.60	12.64
22	4.84	7.38	10.84	13.19
23	3.33	6.17	9.25	13.46
24	4.29	7.14	10.19	12.56
25	5.10	6.86	10.01	10.75
26	4.76	6.70	9.24	13.86
27	3.77	6.60	8.85	14.77
28	4.52	7.12	10.86	13.79
29	3.46	6.67	9.41	13.96
30	4.76	7.35	11.32	14.76
31	4.97	7.60	10.23	14.57
Average	4.41	6.43	9.28	12.51

The seed beds at the Roseville substation were sowed April 10 and transplanting was made June 8. The plants were topped August 7 and harvested August 24. The fourth sampling was made the latter date while the first, second and third samplings were made July 24, August 3, and August 13, respectively.

TABLE 3.—THE YIELD OF TOBACCO OF THE INDIVIDUAL PLOTS OF TIER III AS DETERMINED AT THE END OF THE CURING PERIOD.

Plot No.	Total yield Pounds per Acre	Percentage of "Wrappers"	Percentage of "Fillers"	Percentage of "Seconds"
1	1743	37	39	24
2	1890	50	36	14
3	1964	70	21	9
4	1617	73	25	2
5	1502	71	28	1
6	1386	65	29	6
7	1533	74	25	1
8	1313	71	27	2
9	1481	76	23	1
10	1449	69	30	1
11	1470	75	24	1
12	1670	75	24	1
13	1817	80	20	0
14	1743	77	22	1
15	1418	76	23	1
16	1691	75	24	1
17	1974	74	25	1
18	2048	80	20	0
19	1355	64	33	3
20	1701	72	28	0
21	1869	70	29	1
22	1917	67	32	1
23	1869	71	26	3
24	1764	76	21	3
25	1901	73	23	4
26	1869	82	17	1
27	1691	63	23	14
28	2037	74	23	3
29	1439	59	32	9
30	1712	74	25	1
31	2091	75	25	0

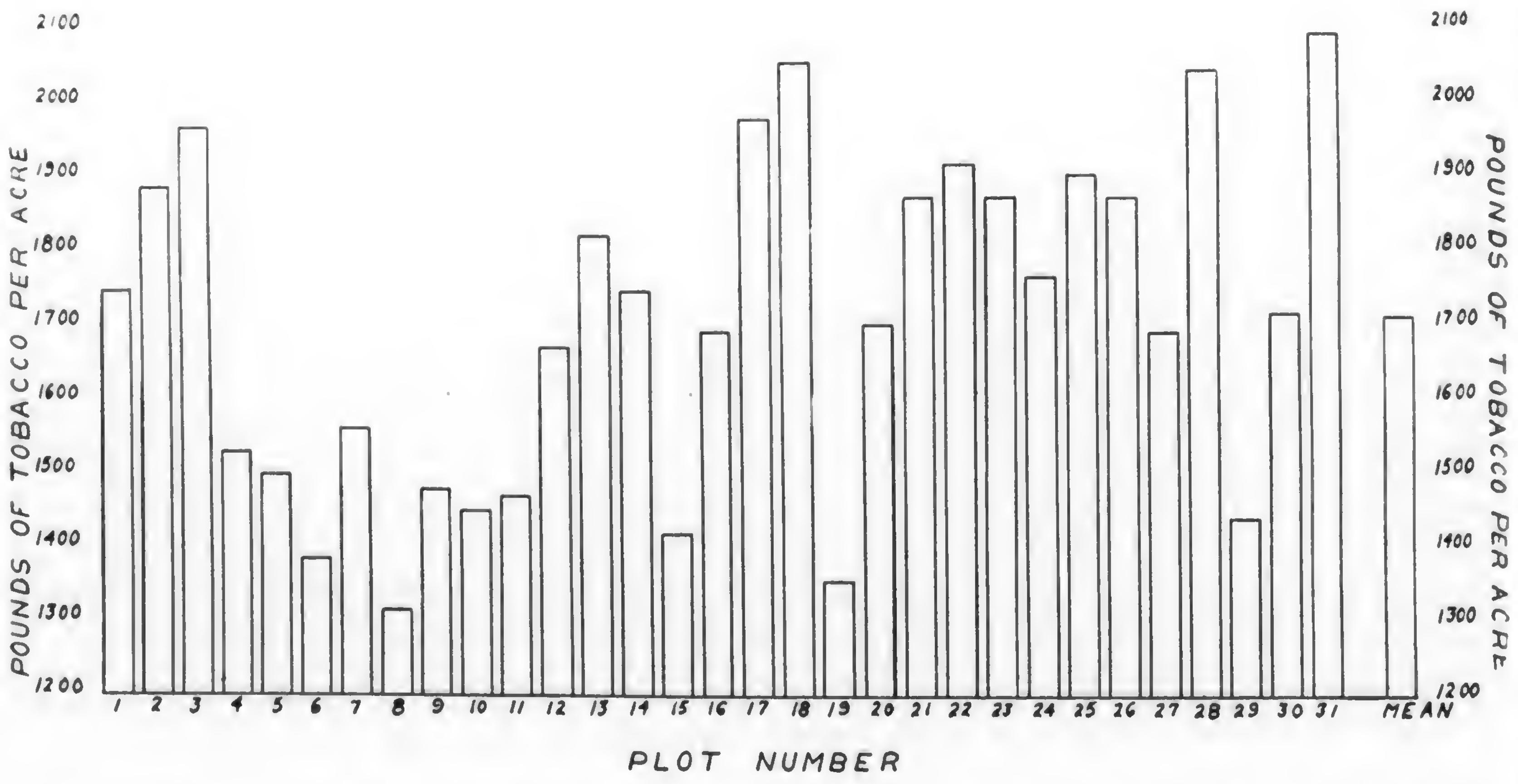

FIG. 2.—THE RELATIVE YIELD OF TOBACCO FROM THE 31 PLOTS OF TIER III FOR THE YEAR 1934

From the standpoint of quantity and distribution, the rainfall for 1934 at the Roseville substation was less than normal for this area. The observed precipitation as compared to the normal was as follows:

Month	*Observed inches*	*Normal inches*
March	2.12	3.53
April	0.95	3.55
May	2.73	3.53
June	3.49	4.22
July	4.28	4.54
August	3.24	4.90

From transplanting to harvesting a precipitation of 11.35 inches was recorded; 3.01 inches of which came after the plants were topped.

TABLE 4.—THE NITROGEN CONTENT OF THE 1934 TOBACCO CROP AS BASED ON SAMPLES TAKEN FROM THE EXPERIMENTAL PLOTS AT DIFFERENT INTERVALS DURING THE GROWING SEASON. THE RESULTS ARE EXPRESSED ON THE AVERAGE MOISTURE-FREE WEIGHT OF THE SIXTH LEAF.

Plot No.	Sampling							
	First		Second		Third		Fourth	
	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf
1	5.13	286	4.82	347	4.69	460	4.54	557
2	4.71	248	5.21	314	4.74	470	4.88	686
3	5.19	241	4.95	331	5.00	591	4.27	509
4	4.25	205	4.56	283	5.20	499	4.43	573
5	4.56	164	4.87	244	4.76	405	4.01	466
6	4.36	186	4.97	247	5.16	430	4.31	530
7	5.08	233	4.91	303	5.31	453	4.41	503
8	4.49	192	4.80	277	5.45	370	4.55	460
9	4.79	260	4.94	290	5.30	425	4.47	432
10	4.26	186	4.72	275	4.78	424	3.77	407
11	5.08	183	4.78	251	5.05	422	4.43	535
12	4.28	233	4.77	331	4.84	407	4.33	510
13	5.04	222	4.97	336	5.10	498	4.60	587
14	4.21	171	4.94	342	5.00	445	5.25	638
15	4.85	208	4.61	315	4.85	367	4.79	510
16	4.50	188	4.85	321	5.29	380	4.81	580
17	5.11	188	4.86	341	5.21	464	4.81	612
18	4.46	229	5.01	297	5.10	577	4.55	638
19	5.23	177	4.64	246	5.04	454	4.93	580
20	4.29	181	4.75	332	4.80	451	4.54	540
21	4.83	178	4.60	253	4.74	407	4.71	596
22	4.53	220	4.69	346	4.93	535	4.56	602
23	5.10	170	4.83	298	5.03	465	4.81	648
24	4.40	189	4.75	339	5.09	518	4.40	553
25	4.87	248	4.83	338	4.94	494	4.73	508
26	4.51	214	5.15	345	5.13	474	4.38	607
27	4.88	184	4.54	299	5.10	452	4.60	679
28	4.45	201	4.75	339	5.16	560	4.33	597
29	5.30	183	5.21	348	5.11	481	4.83	675
30	4.51	215	5.06	372	5.27	597	4.98	735
31	5.01	249	4.91	373	5.00	511	4.66	679
Mean	4.72	207.2	4.85	312.0	5.04	467.3	4.57	575.2

Experimental

From the dry weight of the samples obtained the average weight of an individual leaf was calculated for each sampling. The results obtained are given in Table 2.

Under the supervision of Otto Olson, Federal representative at the Roseville station, the total yields from the different plots were made at the end of the curing season and the tobacco was graded according to the usual procedure into "wrappers," "fillers," and "seconds." The results are given in Table 3 while a graphical presentation of the results on total yield may be found in Fig. 2.

TABLE 5.—THE PHOSPHORUS CONTENT OF THE TOBACCO CROP AS BASED ON SAMPLES TAKEN FROM THE EXPERIMENTAL PLOTS AT DIFFERENT INTERVALS DURING THE GROWING SEASON. THE RESULTS ARE EXPRESSED ON THE AVERAGE MOISTURE-FREE WEIGHT OF THE SIXTH LEAF.

Plot No.	Sampling							
	First		Second		Third		Fourth	
	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf
1	.319	17.7	.269	19.4	.250	24.5	.241	29.6
2	.269	14.2	.295	17.8	.251	24.8	.260	36.6
3	.327	15.2	.265	17.7	.268	31.7	.224	26.6
4	.220	10.6	.233	14.4	.257	24.6	.230	29.8
5	.271	9.7	.240	12.0	.226	19.2	.214	24.8
6	.216	9.3	.266	13.2	.271	22.6	.209	25.7
7	.295	13.5	.239	14.8	.257	21.9	.212	24.2
8	.218	9.3	.245	14.1	.280	19.0	.209	21.1
9	.250	14.7	.260	15.2	.259	20.8	.213	20.6
10	.231	10.1	.265	15.4	.241	21.4	.207	22.4
11	.304	10.9	.242	12.7	.243	20.3	.234	28.3
12	.220	11.9	.253	17.6	.242	20.4	.219	25.8
13	.295	13.0	.246	16.6	.243	23.7	.215	27.5
14	.227	9.2	.263	18.2	.252	22.5	.237	28.9
15	.307	13.2	.266	18.2	.276	20.9	.237	25.2
16	.258	10.8	.263	17.4	.259	18.6	.247	29.7
17	.329	12.1	.270	18.9	.272	24.2	.232	29.5
18	.244	12.5	.275	16.3	.265	30.0	.229	32.1
19	.346	11.7	.277	14.7	.261	23.5	.226	26.5
20	.240	10.1	.262	18.3	.267	25.1	.238	28.4
21	.301	11.1	.264	14.6	.279	24.0	.238	30.1
22	.265	12.9	.269	19.9	.279	30.3	.227	30.0
23	.341	11.4	.271	16.7	.283	26.2	.233	31.4
24	.255	10.9	.284	20.3	.294	30.0	.227	28.5
25	.312	15.9	.291	19.9	.279	27.9	.238	25.6
26	.255	12.1	.291	19.5	.289	26.7	.237	32.9
27	.358	13.5	.272	17.9	.290	25.6	.249	36.8
28	.267	12.1	.289	20.6	.324	35.2	.234	32.3
29	.341	11.8	.280	18.7	.286	26.9	.249	34.8
30	.286	13.6	.293	21.5	.287	32.5	.246	36.4
31	.281	13.9	.283	21.5	.276	28.3	.230	33.5
Mean	.279	12.2	.267	17.2	.268	24.9	.230	28.9

The percentage of nitrogen in all samples obtained was determined and the total quantity absorbed by a single leaf was calculated. Table 4 shows the percentage of nitrogen in the dry matter and likewise the total quantity of nitrogen found in a single leaf.

In like manner the percentage of phosphorus and the total quantity of this element found in a single leaf was calculated. These results are given in Table 5. Similar results for the element potassium are given in Table 6.

A graphical presentation of the data obtained showing the variation in the percentages of nitrogen, phosphorus, and potassium is given in Figs. 3, 4, 5, and 6, while the data on the quantity of these elements absorbed during the growing season are given in Figs. 7, 8, 9, and 10.

Discussion of Results

Investigations concerned with soil and plant interrelationships, especially when conducted under field conditions, often yield results which are difficult to evaluate. The reason for this is that many of the factors involved are not under the direct control of the investigator. The experiments herein described are no exception to the general rule, and it is necessary to repeat such work at frequent intervals in order to arrive at certain definite conclusions. In the meantime, however, it may be possible to note certain trends from the data obtained, which may prove to be of practical significance. In describing the results of the present investigations, attention will be called from time to time to certain factors which appear to influence the absorption of certain mineral nutrients and which therefore affect the growth and development of the plants studied.

TABLE 6.—THE POTASSIUM CONTENT OF THE 1934 TOBACCO CROP AS BASED ON SAMPLES TAKEN FROM THE EXPERIMENTAL PLOTS AT DIFFERENT INTERVALS DURING THE GROWING SEASON. THE RESULTS ARE EXPRESSED ON THE AVERAGE MOISTURE-FREE WEIGHT OF THE SIXTH LEAF.

Plot No.	Sampling							
	First		Second		Third		Fourth	
	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf	Per cent	Mgm. per leaf
1	3.11	173	2.54	183	1.82	179	1.74	213
2	3.23	170	2.88	174	2.32	230	2.53	355
3	3.76	175	3.52	236	3.45	407	2.49	296
4	4.68	226	4.43	275	3.54	340	3.37	403
5	3.63	131	3.23	161	3.49	297	3.66	425
6	4.09	175	2.65	132	3.72	310	3.49	428
7	4.16	191	3.36	208	2.85	243	3.33	380
8	3.54	151	3.57	206	3.48	237	3.46	350
9	4.27	232	3.40	200	3.41	274	3.25	314
10	3.35	147	2.97	173	2.92	259	3.04	329
11	3.33	120	2.72	143	2.99	251	3.19	385
12	4.53	247	3.60	250	3.09	259	3.01	356
13	4.46	196	2.74	252	3.98	389	3.88	515
14	3.76	152	3.22	223	2.97	265	2.92	343
15	3.81	164	2.72	186	4.04	306	3.01	320
16	2.38	99	3.06	203	2.79	200	2.77	334
17	3.48	128	3.28	230	2.85	253	2.98	380
18	3.71	190	3.56	211	3.32	376	3.46	484
19	3.17	107	2.58	137	2.58	233	2.69	316
20	3.59	151	3.10	216	3.66	344	3.24	386
21	3.74	138	3.26	179	3.66	315	2.69	340
22	5.05	245	3.67	271	4.30	467	3.65	482
23	4.21	140	3.55	219	4.06	375	3.65	491
24	4.80	206	4.35	311	3.68	375	3.48	438
25	3.97	202	3.68	252	3.88	388	3.55	382
26	4.92	234	4.21	282	4.30	397	4.24	587
27	4.12	155	4.34	286	3.93	348	3.51	519
28	4.60	208	4.35	310	4.64	504	4.41	609
29	4.06	140	3.70	247	3.10	292	2.96	414
30	4.36	208	4.47	329	3.86	437	3.51	518
31	3.78	188	3.85	293	3.75	383	3.62	528
Mean	3.92	173.8	3.47	225.1	3.43	320.4	3.25	407.1

The Effect of the Soil

While the Hagerstown soils are naturally quite fertile, their productivity is not of uniform distribution. The inherent fertility of soils found on the experimental farm varies considerably. From a study of the check plots, as shown in Fig. 2, it is quite apparent that the soil near plots 5, 10, and 15 is less fertile than soil near plots 20, 25, and 30. This is especially evident by the yield of tabacco obtained from plot 25. These variations in normal fertility must obviously affect the chemical composition of the plants grown thereon, and therefore influence both yield and quality. These soils, however, respond to fertilizer treatment, as the inherent fertility is quite low. This fact is established by the low yields obtained from plots 19 and 29 which received no fertilizer treatment.

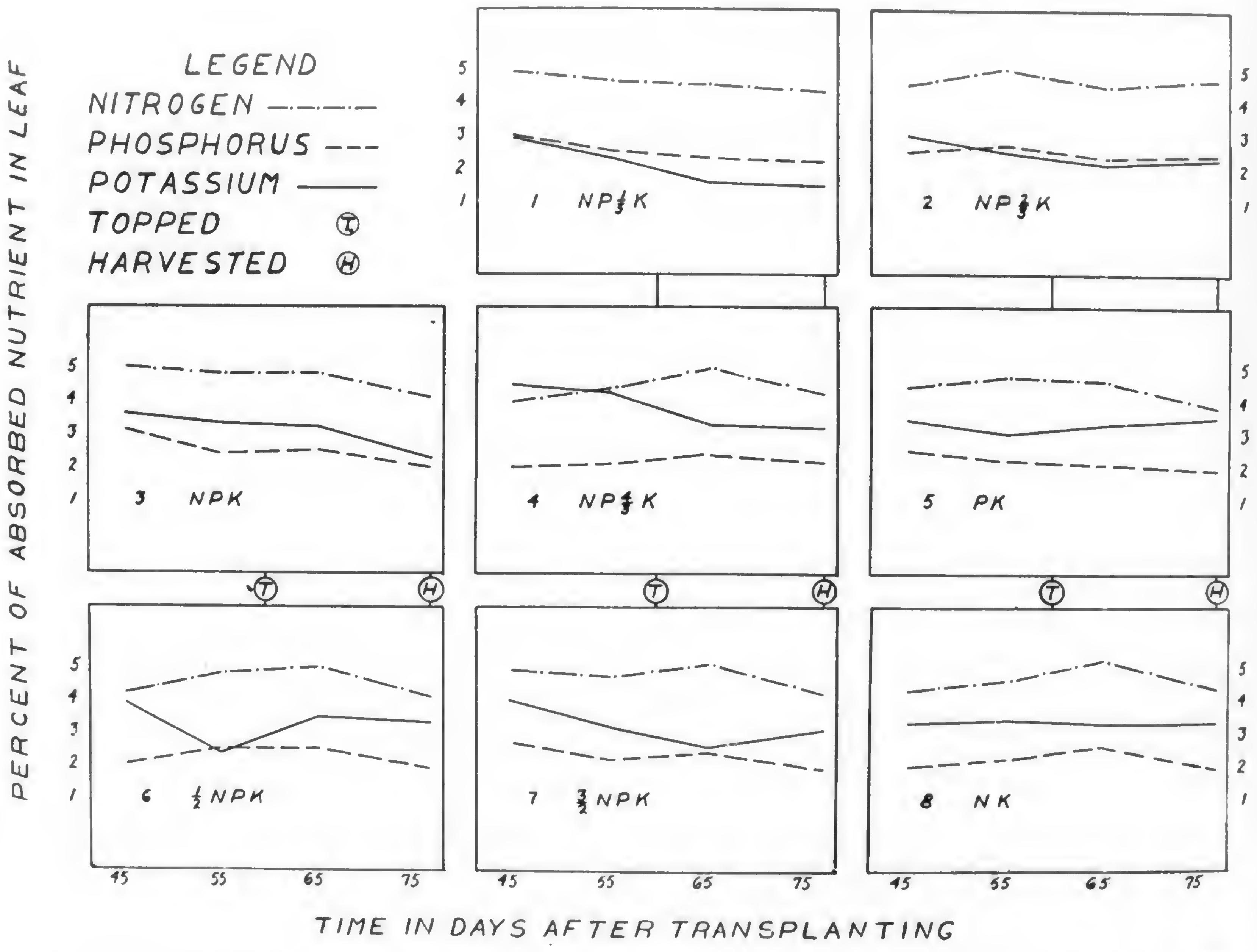

FIG. 3.—THE N, P, AND K CONTENT OF PLANTS FROM PLOTS 1 TO 8 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Percentages are based on the dry matter of the sixth leaf. To obtain absolute values for P divide by 10.

The Effect of Nitrogen

Plots 3, 5, 6, and 7 received varying quantities of nitrogen in the fertilizer applied, the concentration of phosphorus and potassium remaining constant as indicated by the following data:

Plot No.	Quantity of fertilizer applied	Formula	Yield of tobacco		Nitrogen content of mature leaves
	Pounds per acre		Pounds per acre	Percentage of wrappers	Percentage
5	1,000	0-8-12	1,502	71	4.01
6	1,000	3-8-12	1,386	65	4.31
3	1,000	6-8-12	1,964	70	4.27
7	1,000	9-8-12	1,533	74	4.41

From the standpoint of total yield and the production of wrappers it appears that the treatment of plot 3 is superior to the treatments of the other plots of this group.

The effects of nitrogen on the growth and development of tobacco are not only governed by the balance of the nutrient material present but likewise by the form of nitrogen used. In this connection it may be stated that in the fertilizer mixture applied to plot 3, two-thirds of the nitrogen was in the form of cottonseed meal and one-third in the form of nitrate of soda. In the mixture applied to plot 14, one-third of the nitrogen was in the form of cottonseed meal and two-thirds in the form of nitrate of soda. The data obtained, as indicated below, indicate that cottonseed meal, from the stand-

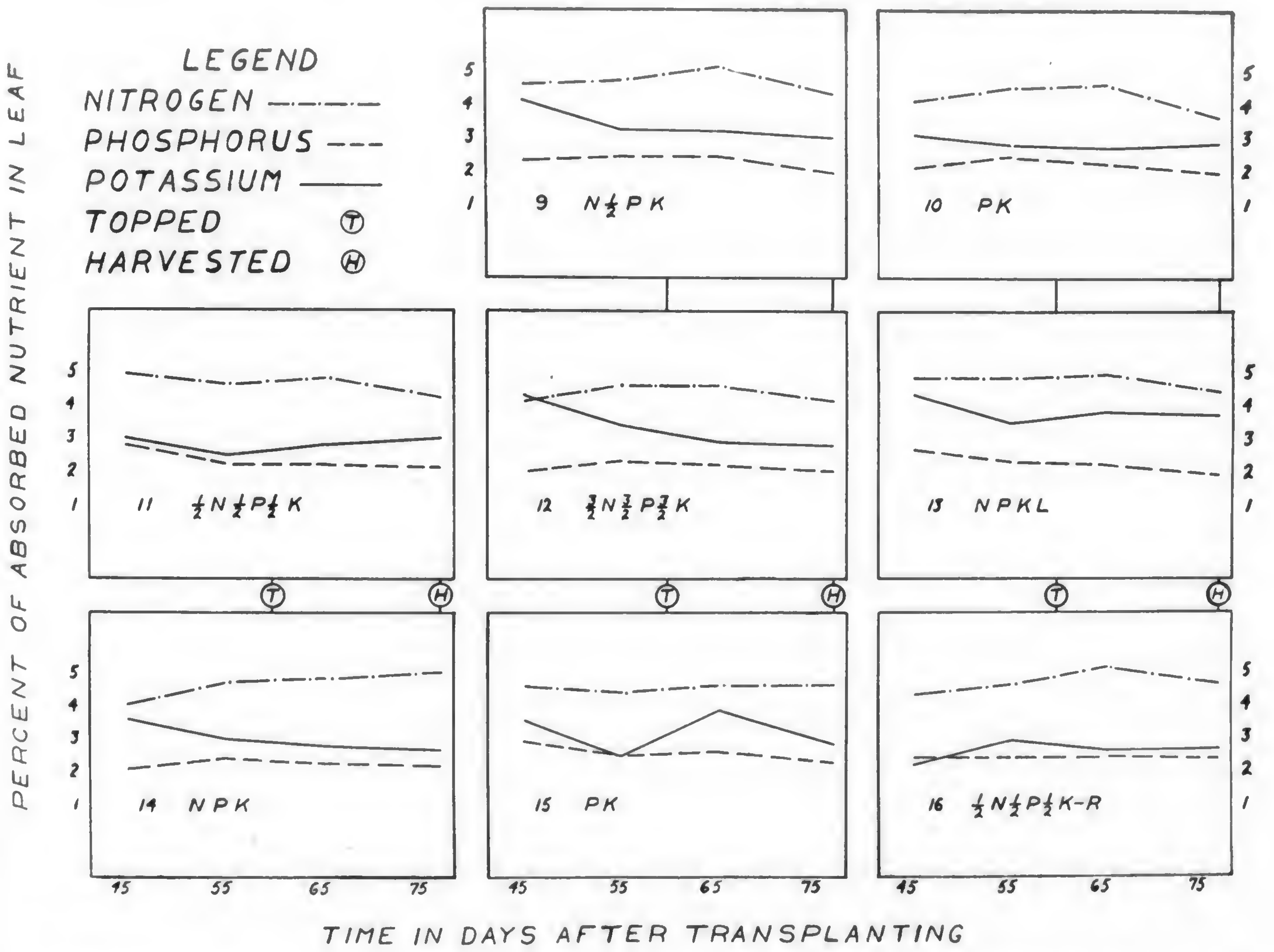

FIG. 4.—THE N, P, AND K CONTENT OF PLANTS FROM PLOTS 9 TO 16 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Percentages are based on the dry matter of the sixth leaf. To obtain absolute values for P divide by 10.

Plot No.	Quantity of fertilizer applied	Formula	Yield of tobacco		Nitrogen content of mature leaves
	Pounds per acre		Pounds per acre	Percentage of wrappers	Percentage
3	1,000	6-8-12	1,964	70	4.27
14	1,000	6-8-12	1,743	77	5.25

point of yield, is more satisfactory as a carrier of nitrogen than nitrate of soda. In the case of a slowly decomposing organic material, such as cottonseed meal, however, a certain quantity of readily available mineral nitrogen, such as nitrate of soda, should be used at the same time.

As indicated in Fig. 10 it is evident that the absorption of nitrogen, on the whole, occurs gradually during the growing season, although the percentage of nitrogen as indicated by Fig. 6 shows a tendency to fall as maturity is attained.

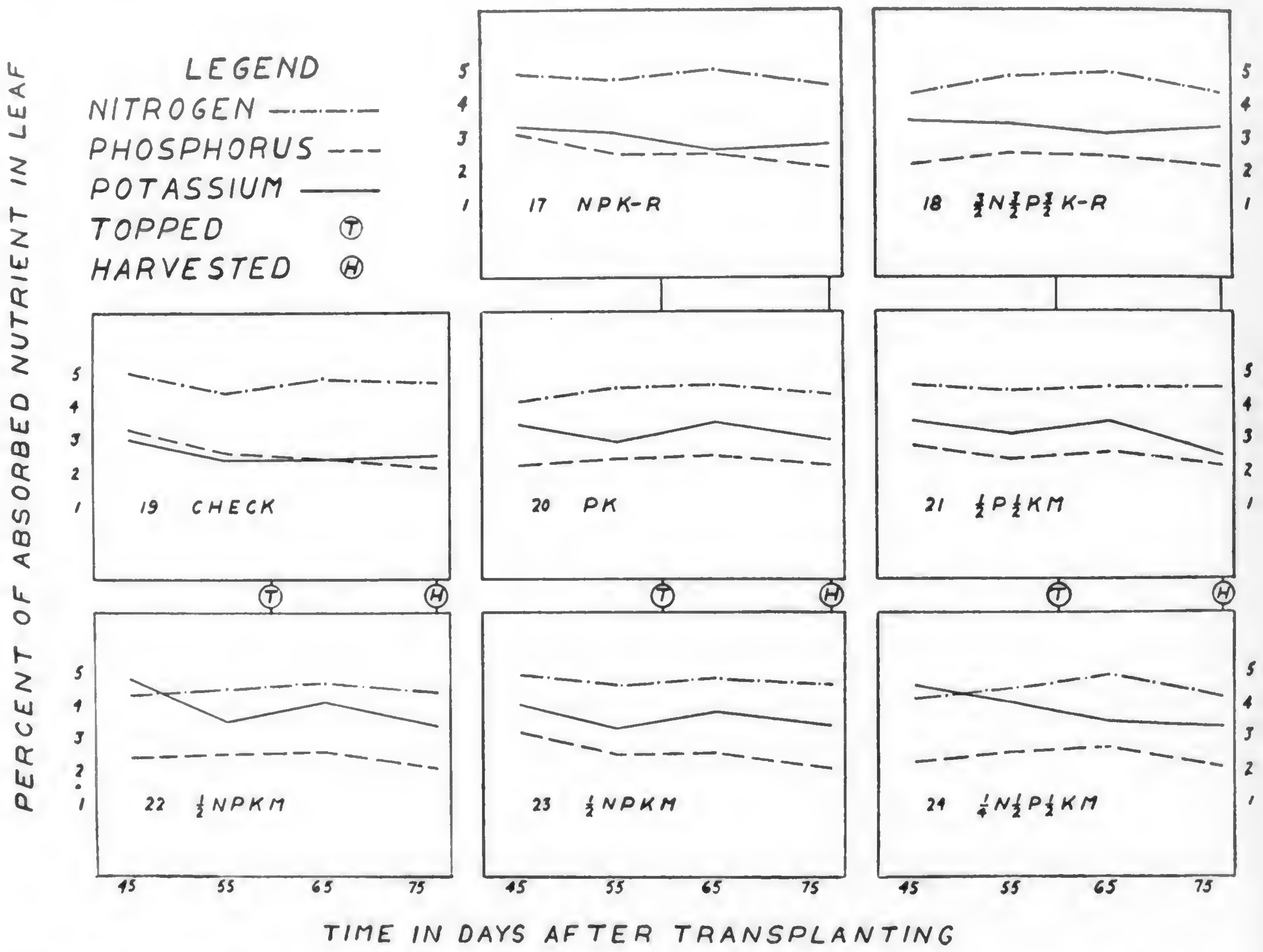

FIG. 5.—THE N, P, AND K CONTENT OF PLANTS FROM PLOTS 17 TO 24 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Percentages are based on the dry matter of the sixth leaf. To obtain absolute values for P divide by 10.
N. B. Plot 21 should be "PKM."

In Table 4 it may be observed that in the first sampling the nitrogen content was highest in those leaves obtained from plots receiving high quantities of nitrogen but lesser quantities of phosphorus and potassium, and where alfalfa preceded the tobacco crop, while the least quantities were from treatments containing small quantities of nitrogen or when nitrogen was supplied mainly as nitrate of soda. In the latter case this was probably due to the fact that nitrate of soda was added one month after transplanting. On the fourth sampling, however, the high quantities were registered from samples taken from plots where alfalfa preceded tobacco, while the least quantities were obtained from plots which received low nitrogen or low phosphorus, and in some cases low potassium additions. It is of interest to note that the highest quantity of nitrogen was obtained in plot 30, where alfalfa preceded tobacco in the rotation and which received 1,000 pounds of an 0-8-12 mixture.

The Effect of Phosphorus

In the fertilizer mixtures applied to plots 3, 8, and 9 the concentration of phosphorus was varied while the concentrations of nitrogen and potassium were held constant, as may be noted from the following data:

Plot No.	Quantity of fertilizer applied	Formula	Yield of tobacco		Phosphorus content of mature leaves
	Pounds per acre		Pounds per acre	Percentage of wrappers	Percentage
8	1,000	6-0-12	1,313	71	.209
9	1,000	6-4-12	1,481	76	.213
3	1,000	6-8-12	1,964	70	.224

These results indicate that the response to phosphorus additions is quite marked, and that a 6-8-12 mixture represents a satisfactory balance of nutrients.

Absorption of phosphorus on the whole, takes place slowly during the growing season as may be noted in Fig. 10, while in Table 5 it may be noted that the first sampling showed that greater quantities were absorbed where nitrogen or potassium was lacking or where alfalfa preceded tobacco in the rotation, while the least quantities were noted in samples from plots which

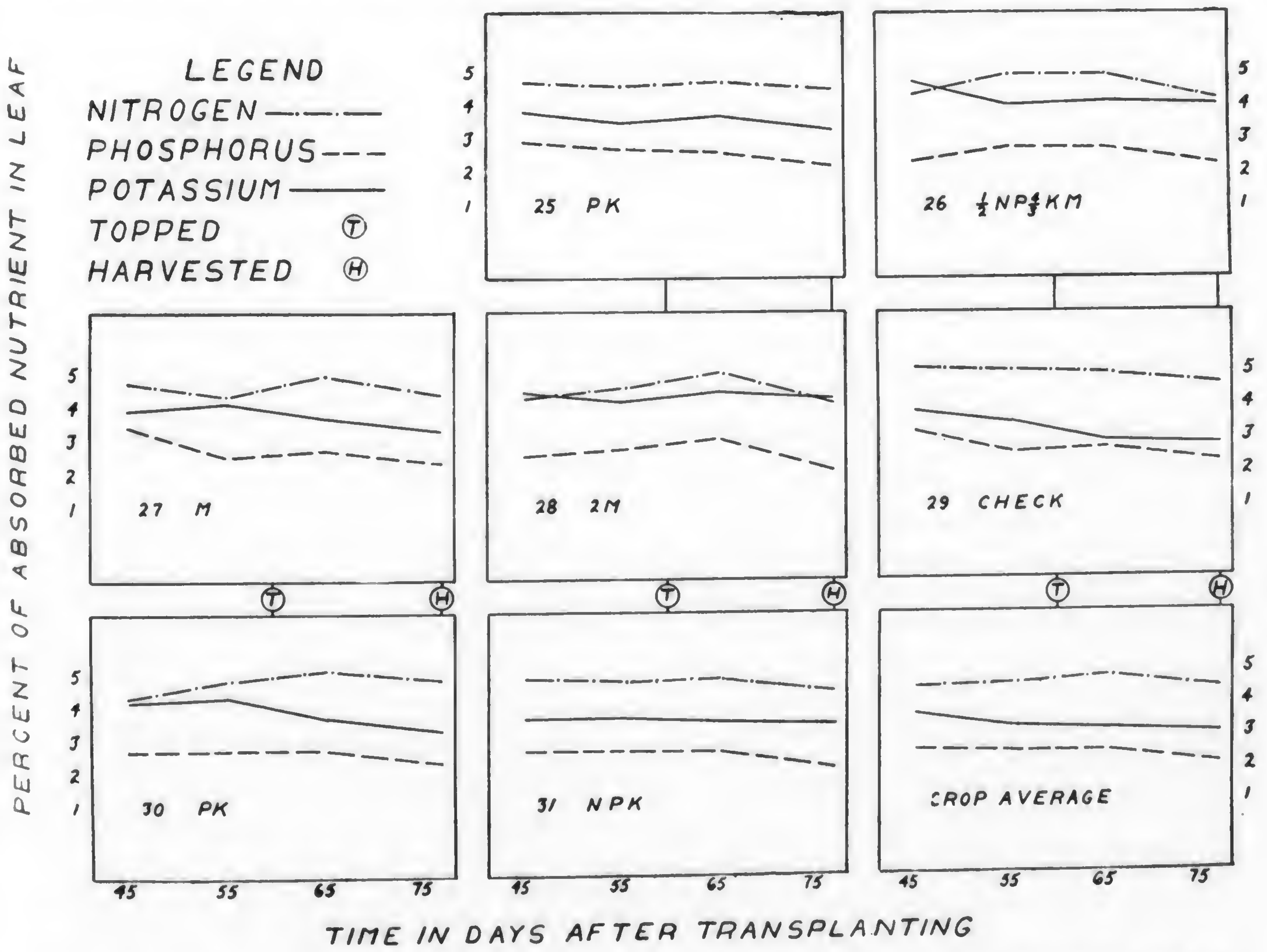

FIG. 6.—THE N, P, AND K CONTENT OF PLANTS FROM PLOTS 25 TO 31 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Percentages are based on the dry matter of the sixth leaf. Averages for the 31 plots are included. To obtain absolute values for P divide by 10.

had received no phosphorus, and where large quantities of nitrogen were applied in the treatment and especially where nitrate of soda was the principal carrier of nitrogen.

In the fourth sampling, the highest quantities of phosphorus were obtained from plots receiving low concentrations of nitrogen and potassium in the fertilizer additions and where alfalfa preceded tobacco in the rotation. The least quantities were obtained from plants receiving mixtures low in phosphorus.

The Effect of Potassium

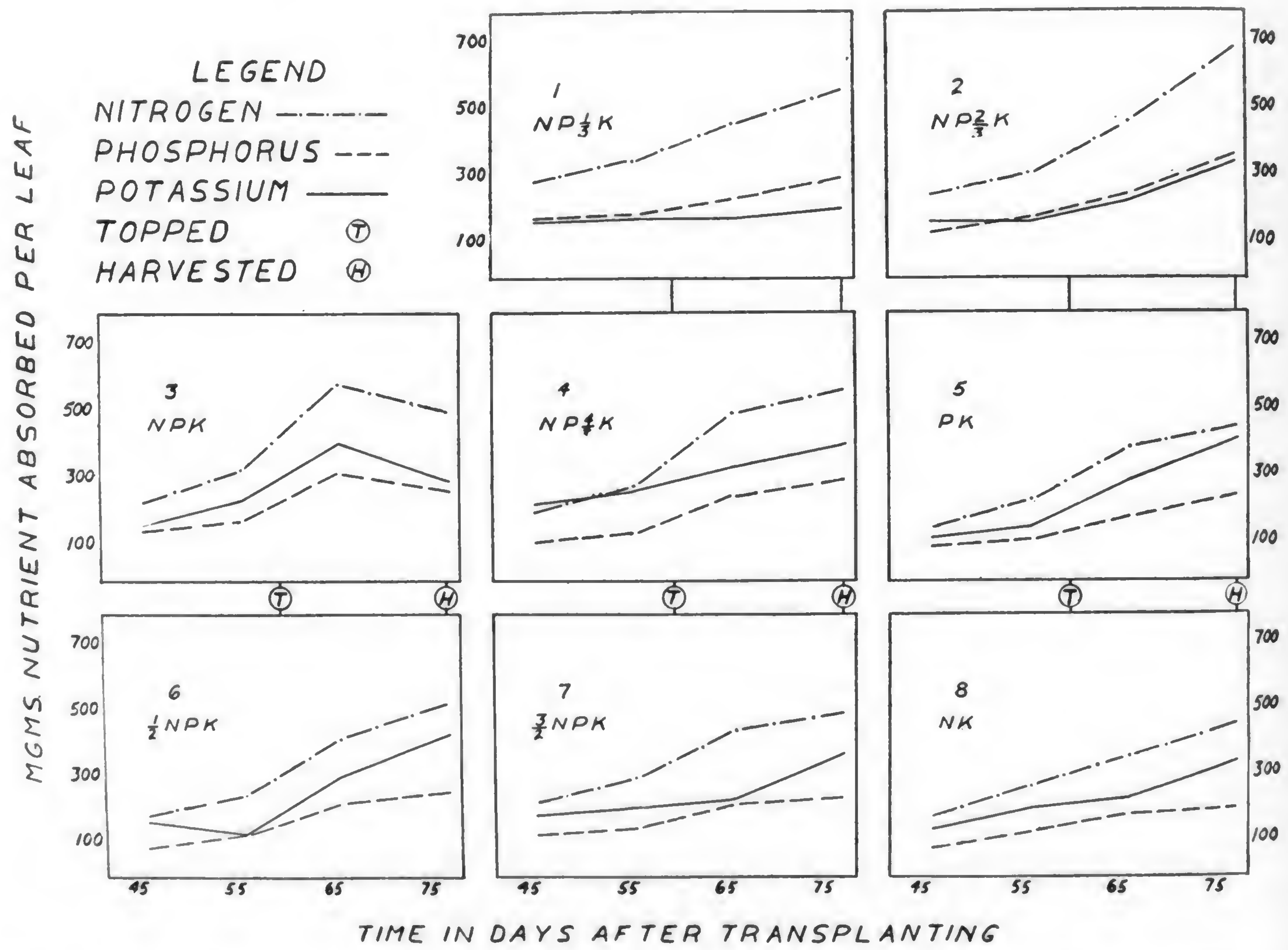

FIG. 7.—THE ABSORPTION OF N, P, AND K BY PLANTS FOR PLOTS 1 TO 8 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Quantities are expressed in milligrams found in the sixth leaf. To obtain absolute values for P divide by 10.

Plots 1, 2, 3, and 4 received varying quantities of potassium while the phosphorus and nitrogen concentrates were held constant. These treatments influenced both yield and production of wrappers as may be noted from the following data:

Plot No.	Quantity of fertilizer applied	Formula	Yield of tobacco		Potassium content of mature leaves
	Pounds per acre		Pounds per acre	Percentage of wrappers	Percentage
1	1,000	6-8-4	1,743	37	1.74
2	1,000	6-8-8	1,890	50	2.53
3	1,000	6-8-12	1,960	70	2.49
4	1,000	6-8-16	1,617	73	3.37

These plots were adjacent to an area which was seriously infected with wild-fire the preceding year. As a result, the disease was contracted before the plants on these plots were mature. Moreover, these plots were of a lower fertility than others some distance away. From the results obtained it appears that potassium was a limiting factor of plant growth and that a balance represented by the fertilizer added to plot 3 was more desirable from the standpoint of yield than any of the others. An increase was noted in the production of wrappers as well. However, a 6-8-16 formula appears undesirable from the standpoint of yield.

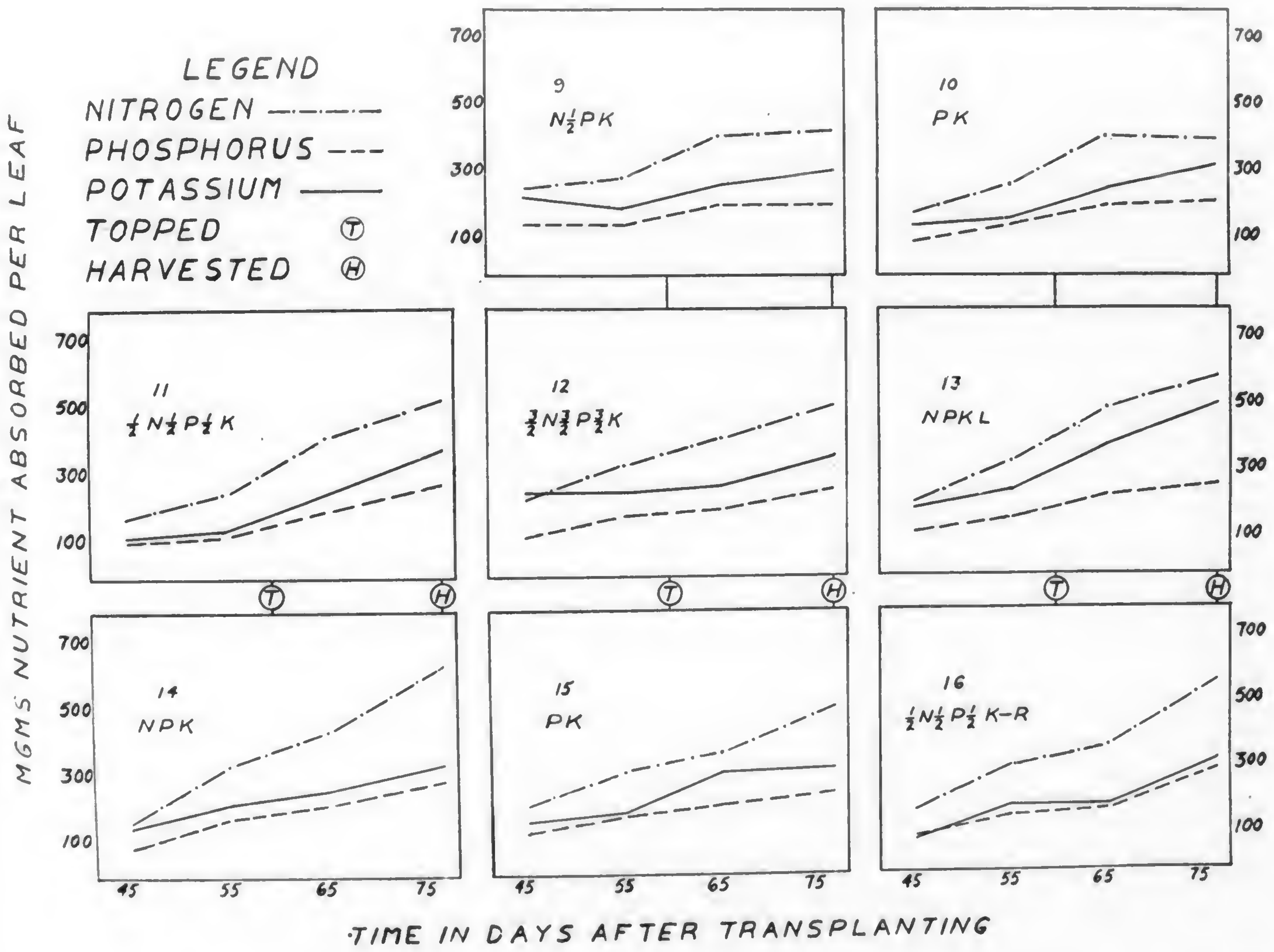

FIG. 8.—THE ABSORPTION OF N, P, AND K BY PLANTS FOR PLOTS 9 TO 16 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Quantities are expressed in milligrams found in the sixth leaf. To obtain absolute values for P divide by 10.

Plot 3 did not show a high concentration of potassium in the fourth sampling which may be attributed, in a measure, to the influence of wildfire, as the third sampling showed this particular plot to contain relatively large quantities of potassium, being exceeded only by plots 20, 22, and 30. In the latter instances, larger quantities of manure were used, alone and in combination with fertilizers containing small quantities of nitrogen when a 0-8-12 fertilizer was used on a plot where alfalfa preceded tobacco in the rotation.

On the whole, the plants containing the larger quantities of potassium were those receiving large quantities of manure, or a high concentration of potash in the fertilizer. On the other hand, the least quantities were found where small quantities of either potassium or phosphorus were applied, the least being found in plot 1.

The absorption of potassium during the growing season is greater than phosphorus but much less than that of nitrogen. This condition may have been different if the precipitation had been greater than that registered.

The Effect of Manure

It should be noted that additions of manure not only serve to increase the organic matter content of the soil but act as a direct fertilizing material as well. An application of manure at the rate of 10 tons per acre carries approximately 100 pounds of nitrogen, 50 pounds of phosphoric acid, and 100 pounds of potash.

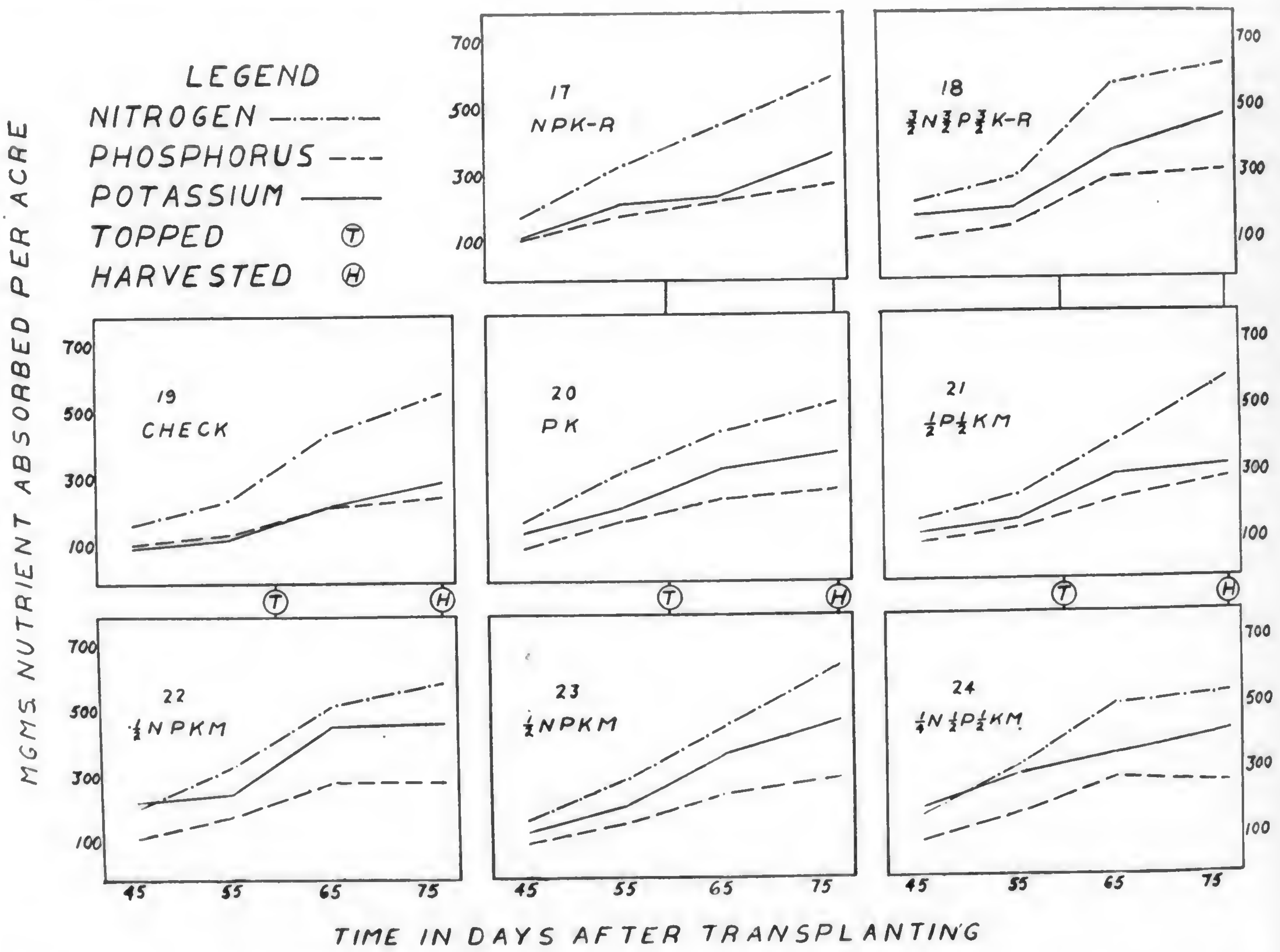

FIG. 9.—THE ABSORPTION OF N, P, AND K BY PLANTS FOR PLOTS 17 TO 24 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Quantities are expressed in milligrams found in the sixth leaf. To obtain absolute values for P divide by 10.

N.B. Plot 21 should be "PKM."

The results obtained with manurial additions with and without supplementary additions may be summarized as follows:

Plot No.	Manure	Fertilizers	Formula	Yield of tobacco		Potassium content of mature leaves
	Tons per acre	Pounds per acre		Pounds per acre	Percentage of wrappers	Percentage
19	(Check)	-----	------	1,355	64	2.69
27	10	-----	------	1,691	63	3.51
21	10	1,000	0-8-12	1,869	70	2.69
25	(Check)	1,000	0-8-12	1,901	73	3.55
24	10	500	3-8-12	1,764	76	3.48
23	10	1,000	3-8-8	1,869	70	3.65
22	10	1,000	3-8-12	1,917	67	3.65
26	10	1,000	3-8-16	1,869	82	4.24
28	20	-----	------	2,037	73	4.41
29	(Check)	-----	------	1,439	59	2.96

An application of manure alone and at the rate of 10 tons per acre does not appear desirable, better results being obtained by using 20 tons. However, an application of 1,000 pounds of an 0-8-12 fertilizer appears to be practically as efficient as a 20-ton, and superior to a 10-ton application of manure.

The effect of potassium when used with manure is as pronounced as in the case of plots 1, 2, 3, and 4 when manure was not used; the highest production of wrappers of the entire tier was obtained from plot 26 receiving 16 units of potash with manure.

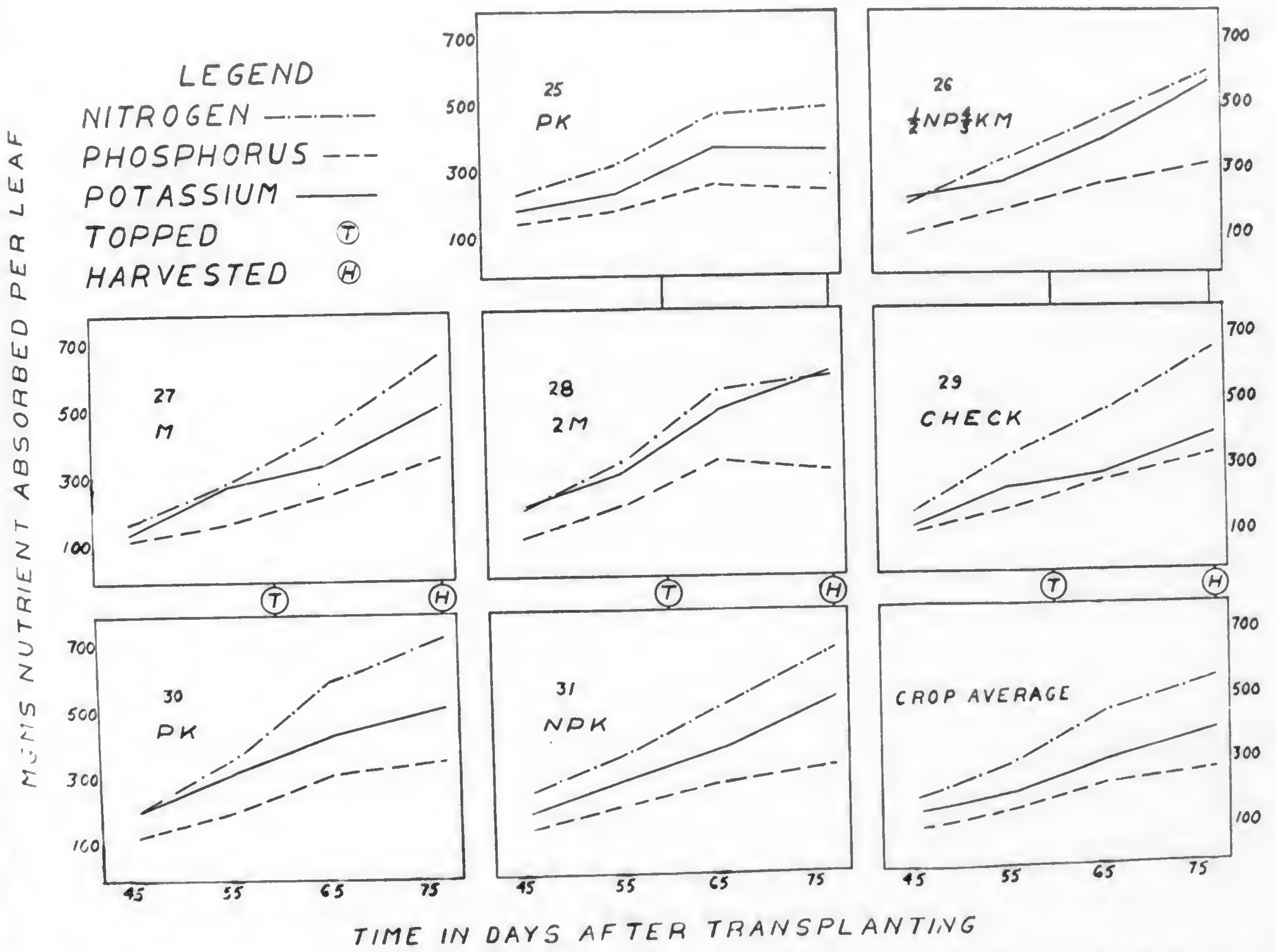

FIG. 10.—THE ABSORPTION OF N, P, AND K BY PLANTS FOR PLOTS 25 TO 31 INCLUSIVE OF TIER III AS DETERMINED AT VARIOUS INTERVALS DURING THE 1934 SEASON

Quantities are expressed in milligrams found in the sixth leaf. To obtain absolute values for P divide by 10.

While the yield and production of wrappers varied materially on the various check plots receiving 1,000 pounds of an 0-8-12 fertilizer per acre, the yields and wrapper production were quite low when no additions were made as in the case of plots 19 and 29, illustrating the necessity of manurial or fertilizer additions.

The Method of Applying Fertilizers and Its Effect

The fertilizer additions made to plots 16, 17, and 18 were applied to the row. On all other plots the fertilizers were applied broadcast. The results obtained in yield and production of wrappers may be summarized as follows:

Plot No.	Quantity of fertilizer used	Formula	Method of application	Yield of tobacco		Potassium content of mature leaves
	Pounds per acre			Pounds per acre	Percentage of wrappers	Percentage
11	500	6-8-12	Broadcast	1,470	75	3.19
16	500	6-8-12	Row	1,691	75	2.77
3	1,000	6-8-12	Broadcast	1,964	70	2.49
17	1,000	6-8-12	Row	1,974	74	2.98
12	1,500	6-8-12	Broadcast	1,670	75	3.01
18	1,500	6-8-12	Row	2,048	80	3.46

The method of applying fertilizer to the row appears to be somewhat more satisfactory from the standpoint of yield than the ordinary method of broadcasting. There is likewise a tendency toward a greater absorption of nutrient materials as may be observed from Tables 4, 5, and 6.

Summary

The results of these preliminary investigations, as based on one year's work, indicate that the absorption of nitrogen, phosphorus, and potassium is rather closely correlated with the quantity at the disposal of the plants.

That clover and alfalfa, when grown in the rotation, add considerably to the quantity of nitrogen available for absorption, is evident from the nitrogen content and relatively high yields of the plots receiving a fertilizer mixture of a 0-8-12 formula when applied at the rate of 1,000 pounds per acre. These yields, moreover, indicate a marked difference between the inherent fertility of the individual plots.

These soils respond to potassium treatments. The best application from the standpoint of yield and quality is 1,000 pounds per acre of a 6-8-12 mixture. There is a response to phosphorus additions as well, the best results being obtained when the same mixture and quantity are used.

Cottonseed meal appears superior to nitrate of soda as a carrier of nitrogen. The yield and production of wrappers, however, appear to be correlated with the formula of the mixture. A 6-8-12 mixture at the rate of 1,000 pounds per acre appears to be most desirable.

While excellent results have been obtained from the use of a 6-8-12 mixture applied at the rate of 1,000 pounds per acre, the method of application is a factor to be taken into consideration. Applications to the row appear superior to the ordinary system of broadcasting.

While apparently large yields were obtained from additions of manure, an application of 1,000 pounds of a 0-8-12 mixture proved equally efficient to a 20-ton application of manure. In any case the supplementing of manure additions with a 3-8-12 mixture at the rate of 1,000 pounds per acre appears desirable.

THE EFFECT OF VITAMIN D INTAKE OF THE HEN ON THE BONE CALCIFICATION OF THE CHICK

BY

R. R. MURPHY, J. E. HUNTER, AND H. C. KNANDEL

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The Effect of Vitamin D Intake of the Hen on the Bone Calcification of the Chick*†

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(Presented at Annual Meeting, August 6-9, 1935)

SEVERAL investigators, Hart and coworkers (1925); Bethke, Kennard, and Sassaman (1927); DeVaney, Munsell, and Titus (1933); and Guerrant, Kohler, Hunter, and Murphy (1935), have shown that the vitamin D potency of the egg yolk is dependent upon the antirachitic intake of the hen producing it. Since a marked relationship has been shown to exist between the antirachitic potency of the egg yolk and the antirachitic intake of the hen producing it, it seemed reasonable to expect a transfer of the vitamin from the hen to the chick which could be measured by the degree of bone calcification.

In previous work (Murphy, Hunter, and Knandel, 1934) in which the quantitative requirements of laying and breeding hens for vitamin D were studied, an attempt was made to measure the degree of transmission of the vitamin from the hen to the chick. The quantity of vitamin D per 100 grams of feed given to nine groups of Single Comb White Leghorn hens, from time of hatch to 76 weeks of age, ranged from 0 to 135 U.S.P. units as shown in Figures 1 and 2. Vitamin D was supplied by adding a cod liver oil concentrate containing 270 U.S.P. units per gram to an all-mash laying ration. Seven of the nine groups were confined in pens devoid of direct sunlight. Two groups had access to sunlight and to limited range. One of the range groups received 34 U.S.P. units of vitamin D per 100 grams of feed. The other range group was fed the laying mash without the addition of the cod liver oil concentrate.

EXPERIMENTAL

Chicks hatched from the hens receiving the various amounts of vitamin D as stated above were brooded for a period of eight weeks in battery brooders housed in a room from which direct sunlight was excluded. The chicks were fed a vitamin D deficient all-mash ration containing 43.5 pounds ground yellow corn, 10 pounds wheat bran, 10 pounds standard wheat middlings, 5 pounds alfalfa leaf meal, 10 pounds meat scraps, 10 pounds of dried milk, 10 pounds ground oats, 1 pound ground limestone, and 0.5 pounds of salt. At four and eight weeks of age 4 representative individuals (2 males and 2 females) were killed and bone ash determined on the right tibiae following the method described by Murphy, Hunter, and Knandel (1934). The split proximal end of each left tibia was photographed to show the degree of calcification.

A study of the bone ash data as presented in Figures 1 and 2 failed to indicate a transmission of vitamin D effect from the hen to the offspring. A careful observation of the bone photographs made at four weeks

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† This work was made possible through the establishment of a fellowship by the National Oil Products Co., Harrison, N.J.

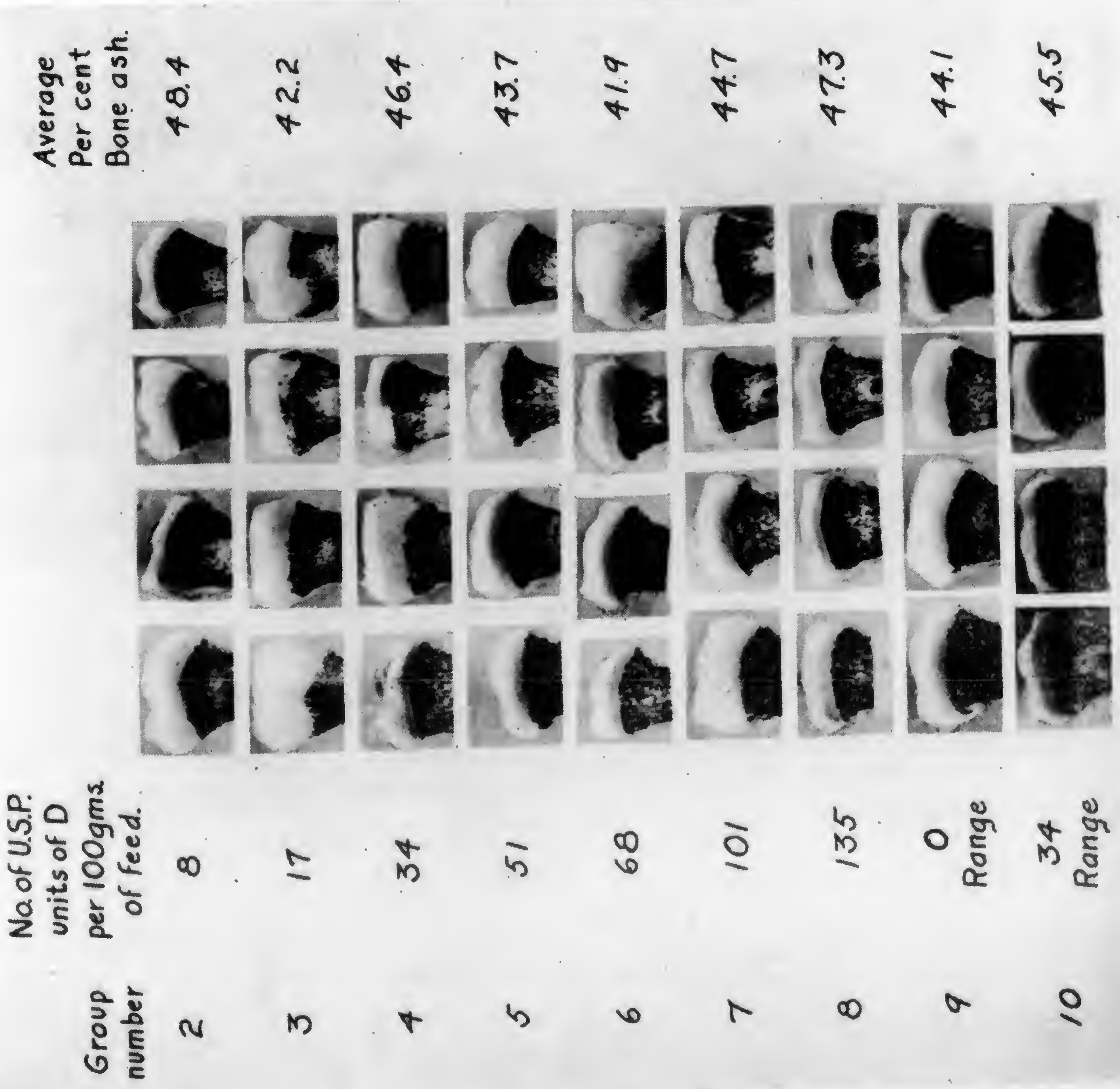


Fig. 1. Internal structure and bone ash of tibiae at four weeks.

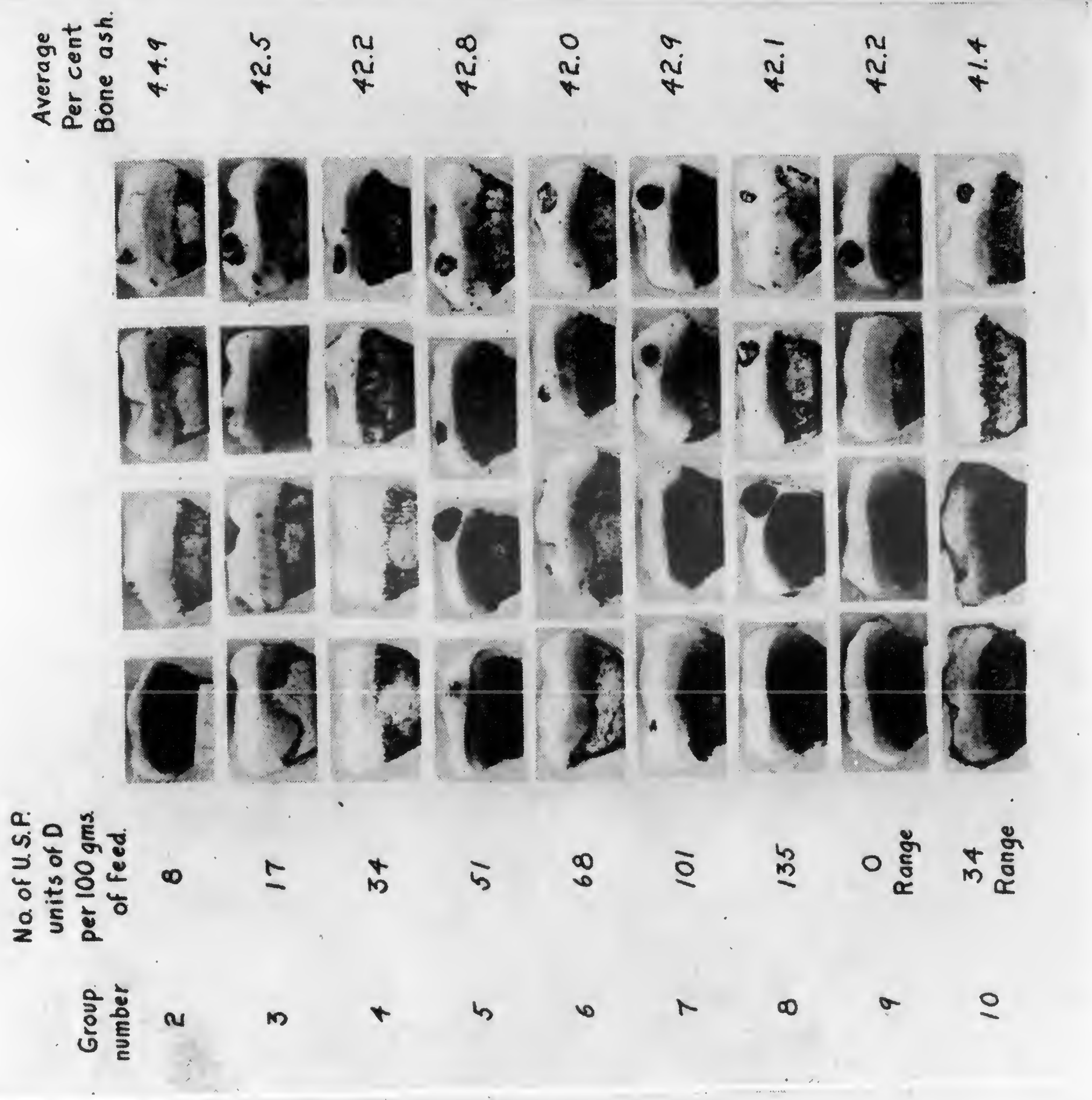
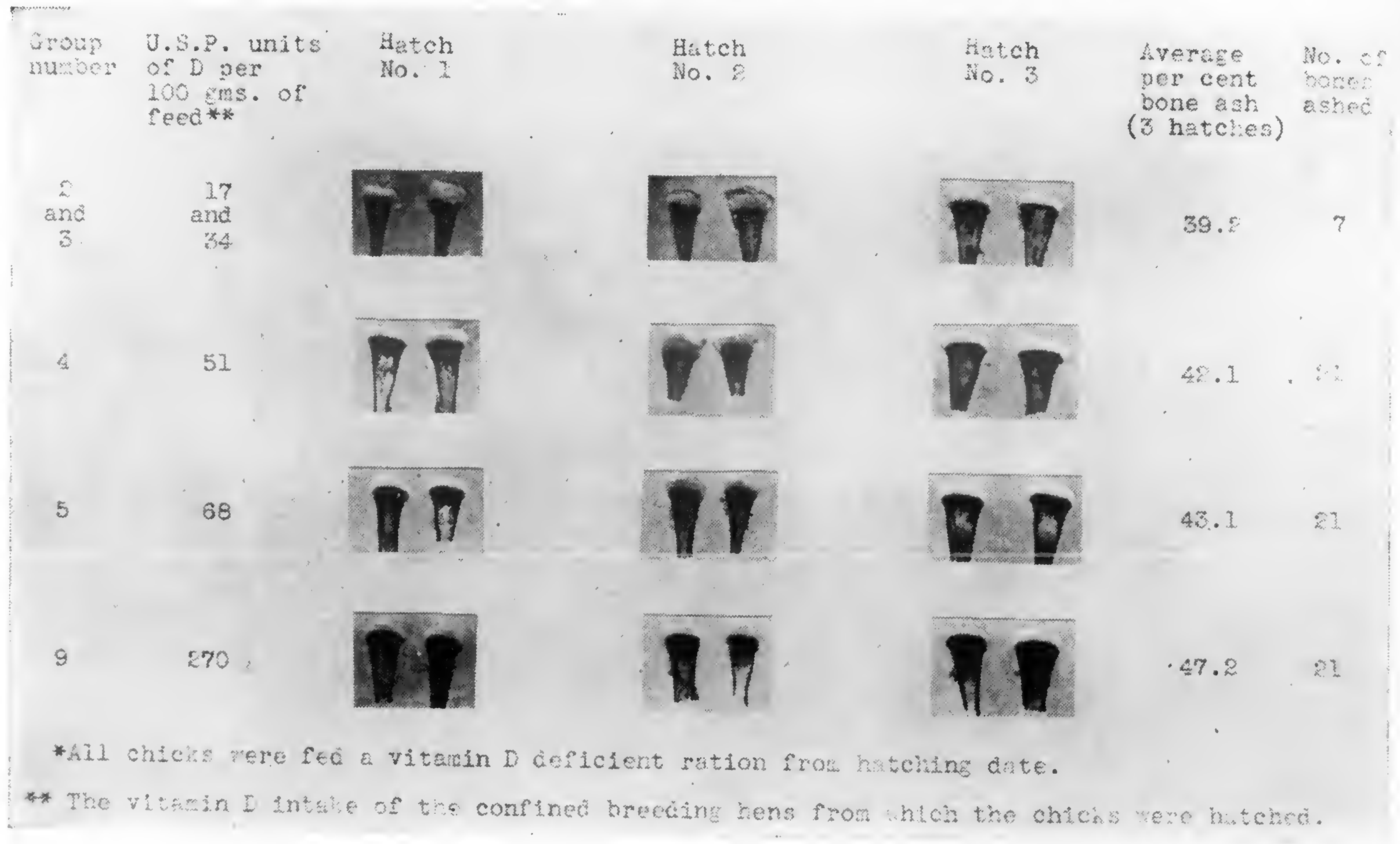


Fig. 2. Internal structure and bone ash of tibiae at eight weeks.

FIG. 3. Internal structure and bone ash of chick* tibiae at one week.

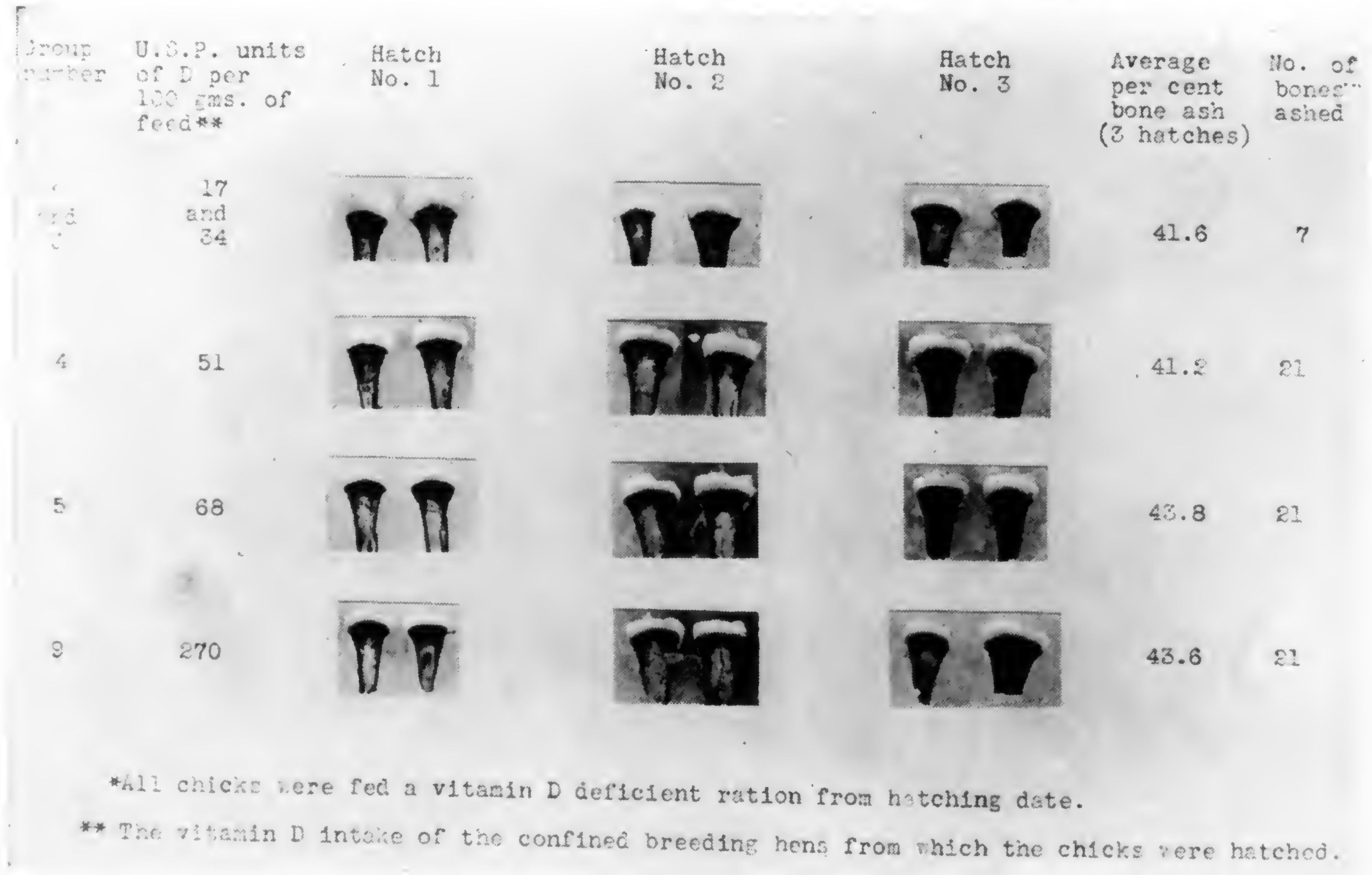


Group number	U.S.P. units of D per 100 gms. of feed**	Hatch No. 1	Hatch No. 2	Hatch No. 3	Average per cent bone ash (3 hatches)	No. of bones ashed
2 and 3	17 and 34				39.2	7
4	51				42.1	[illegible]
5	68				43.1	21
9	270				47.2	21

*All chicks were fed a vitamin D deficient ration from hatching date.

** The vitamin D intake of the confined breeding hens from which the chicks were hatched.

FIG. 4. Internal structure and bone ash of chick* tibiae at two weeks.

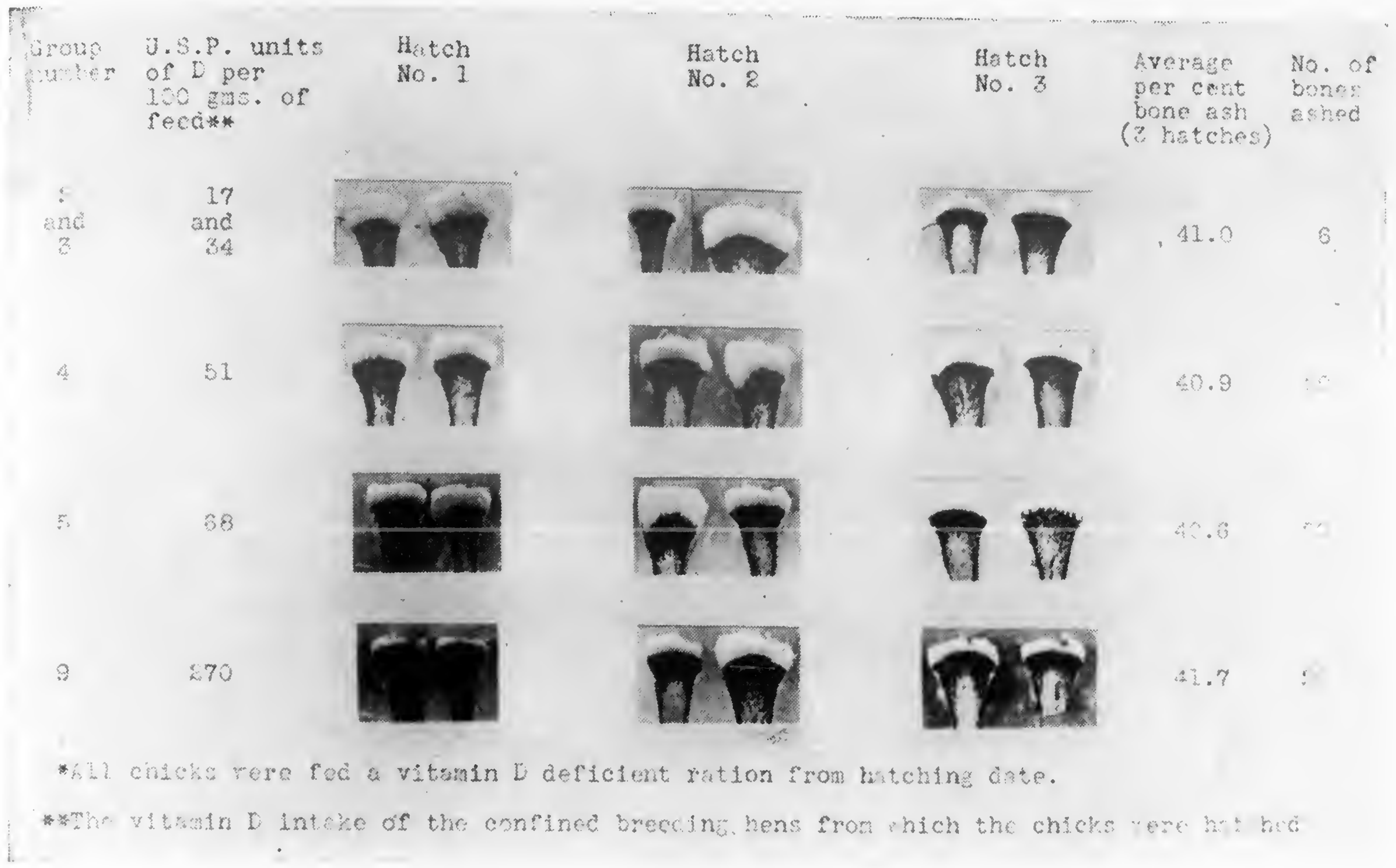


Group number	U.S.P. units of D per 100 gms. of feed**	Hatch No. 1	Hatch No. 2	Hatch No. 3	Average per cent bone ash (3 hatches)	No. of bones ashed
[illegible]	17 and 34				41.6	7
4	51				41.2	21
5	68				43.8	21
9	270				43.6	21

*All chicks were fed a vitamin D deficient ration from hatching date.

** The vitamin D intake of the confined breeding hens from which the chicks were hatched.

FIG. 5. Internal structure and bone ash of chick* tibiae at three weeks.

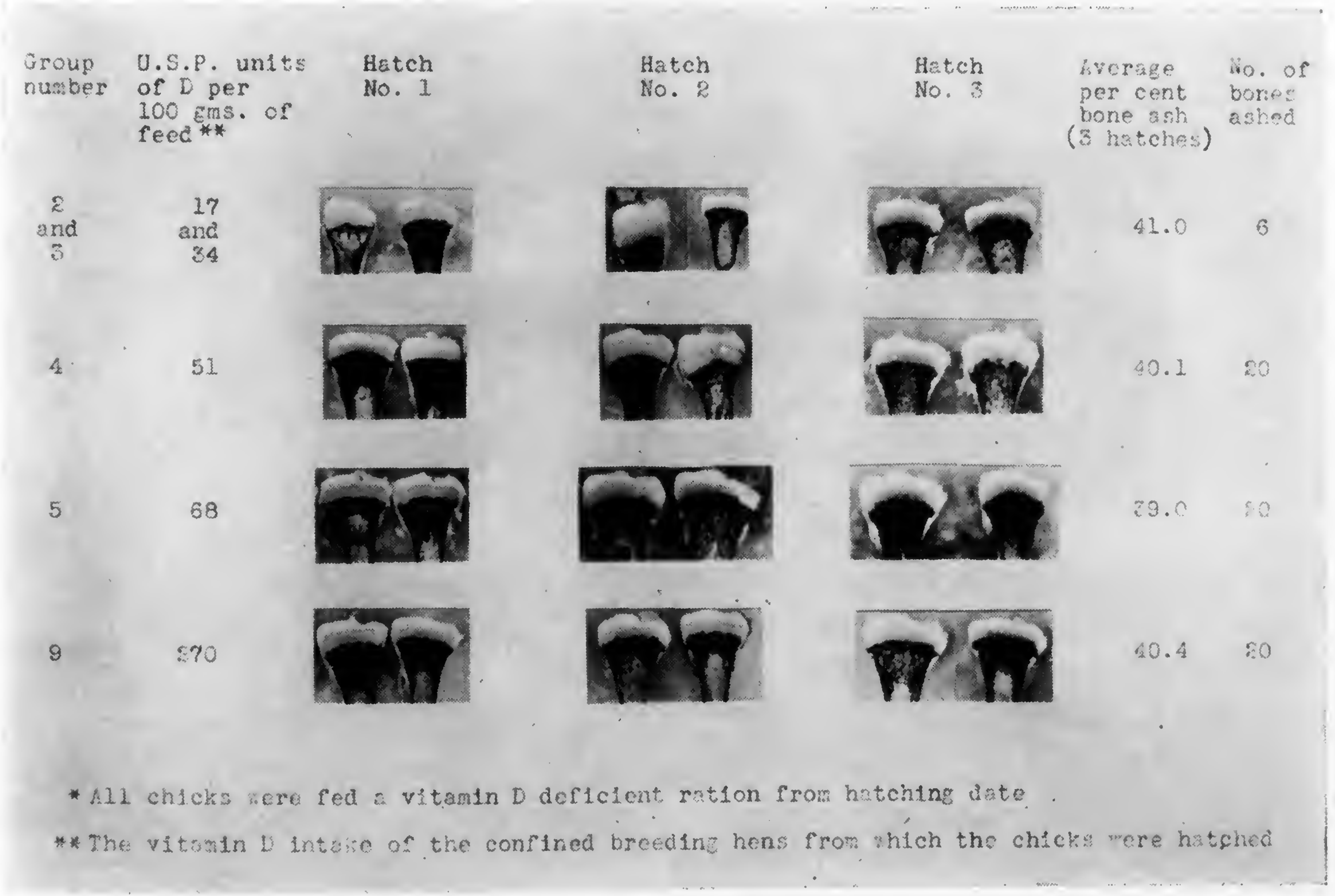


Group number	U.S.P. units of D per 100 gms. of feed**	Hatch No. 1	Hatch No. 2	Hatch No. 3	Average per cent bone ash (3 hatches)	No. of bones ashed
2 and 3	17 and 34				41.0	6
4	51				40.9	[illegible]
5	68				40.6	[illegible]
9	270				41.7	[illegible]

*All chicks were fed a vitamin D deficient ration from hatching date.

**The vitamin D intake of the confined breeding hens from which the chicks were hatched

FIG. 6. Internal structure and bone ash of chick* tibiae at four weeks.



Group number	U.S.P. units of D per 100 gms. of feed **	Hatch No. 1	Hatch No. 2	Hatch No. 3	Average per cent bone ash (3 hatches)	No. of bones ashed
2 and 3	17 and 34				41.0	6
4	51				40.1	20
5	68				39.0	20
9	270				40.4	20

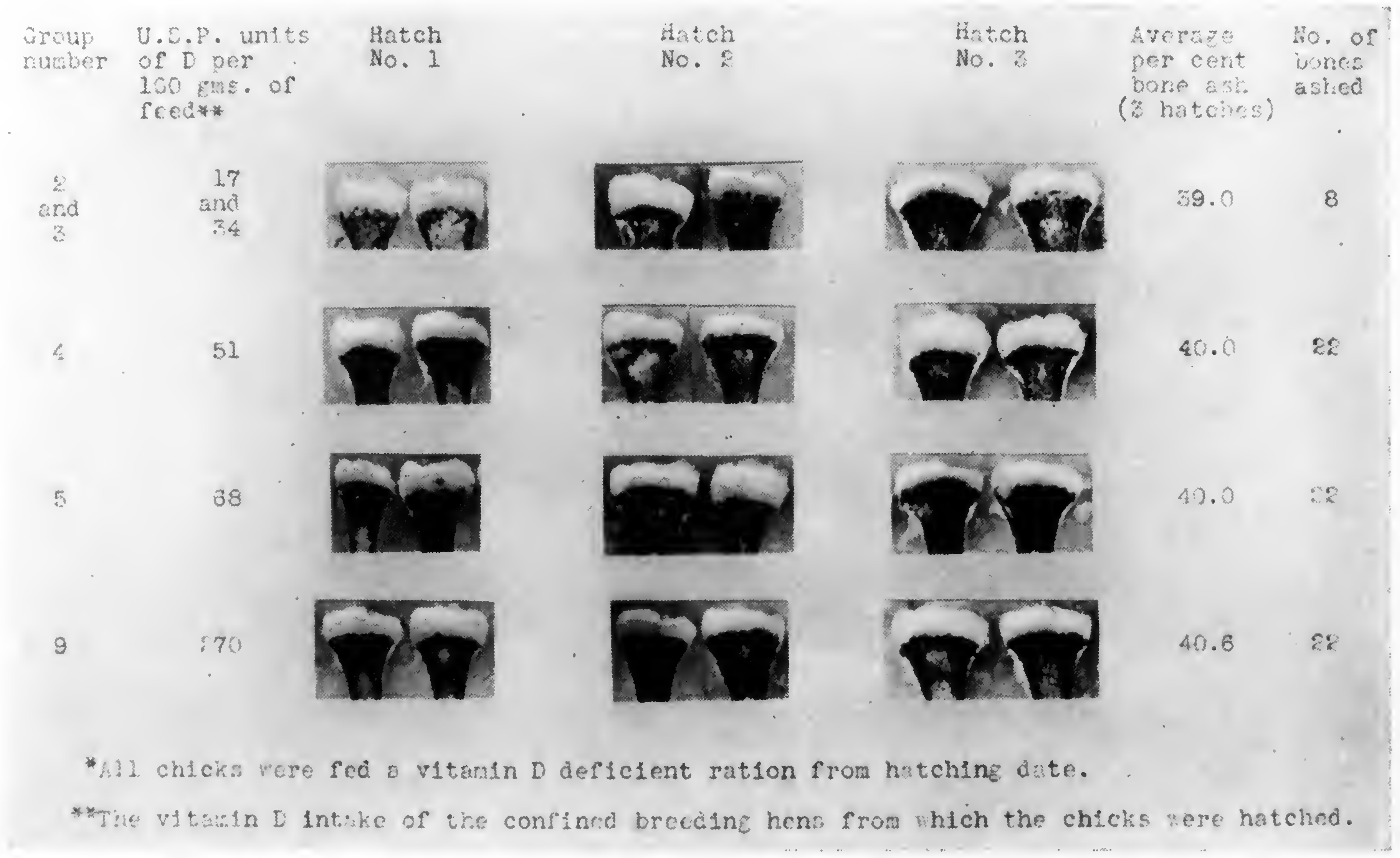
* All chicks were fed a vitamin D deficient ration from hatching date.

**The vitamin D intake of the confined breeding hens from which the chicks were hatched

showed superior calcification in those groups of chicks hatched from hens that received the higher levels of vitamin D in their rations. A study of the bone photographs made at eight weeks failed to reveal an evidence of vitamin D carry over from the hen to the chick. Since evidence showing a transmission of vitamin D effect from the hen to the chick was obtained at four weeks but was not evident when the chicks were eight weeks of age it was thought advisable to make bone ash determinations and bone photographs at weekly intervals during the early brooding period.

A second experiment was conducted in which the vitamin D per 100 grams of feed given to five groups of confined breeding hens, from time of hatch to 76 weeks of age, ranged from 17 to 270 U.S.P. units as shown in Figures 3, 4, 5, 6, and 7. The vitamin D was supplied by adding a cod liver oil concentrate containing 270 U.S.P. units of vitamin D per gram to an all-mash laying ration. Three groups of chicks were hatched from each pen of hens. All chicks from each hatch were marked for identification and brooded together in battery brooders for a period of five weeks. All chicks received the same vitamin D deficient ration as was fed in the first experiment. Every seven days during the brooding period representative individuals were killed and bone ash determined on the right tibiae. Bone photographs were made from each left tibiae.

Fig. 7. Internal structure and bone ash of chick* tibiae at five weeks.



*All chicks were fed a vitamin D deficient ration from hatching date.

**The vitamin D intake of the confined breeding hens from which the chicks were hatched.

Figures 3 4, 5, 6, and 7 show the average percent bone ash of the three hatches together with corresponding photographs of representative bones. Because of the small number of chicks obtained from the hens in groups 2 and 3 these hatches were combined and considered as one group. From a study of bone ash data and bone photographs obtained at the time the chicks were one week old, Figure 3, there appeared to be a direct correlation between the vitamin D intake of the hen and the degree of bone calcification in the resultant chick. At two weeks of age a correlation was still in evidence but not as marked as it was at one week of age (Figure 4). The evidence was still less marked by the time the chicks were three weeks of age. These differences in the degree of bone calcification disappearing at a very early age explains why negative results were obtained in the first experiment at eight weeks. The differences apparent in the bone calcification of the chicks at an early age were perhaps due to the vitamin D reserve of the eggs from which the chicks were hatched and not to differences in the mineral content of the eggs. Hart and co-workers (1925) found that the calcium and phosphorous content of the whites and yolks of eggs laid by hens irradiated with ultra-violet light was not essentially different from those laid by non-irradiated hens.

It is of interest to note (Table 1) the relationship between the bone ash of one-week-old chicks hatched from the various groups of breeding hens and the vitamin D potency (Guerrant, Kohler, Hunter, and Murphy, 1935) of the egg yolks from eggs produced by the same hens. It is apparent that the vitamin D intake of the hen influences the vitamin D content of the egg which in turn has a marked effect on the degree of bone calcification, occurring in chicks during the early growth period.

Table 1.—*A comparison of the percent bone ash of 1 week old chicks with the vitamin D potency of egg yolk*

Group number	U.S.P. units of D per 100 gms. of feed*	Average percent bone ash (1 week)	U.S.P. units of D per gm. of egg yolk†
2	17	39.2	0.32
3	34		0.54
4	51	42.1	0.68
5	68	43.1	0.68
9	270	47.2	5.40

* The vitamin D intake of the confined breeding hens from which the chicks were hatched and from which the samples of egg yolk were taken.

† Guerrant, Kohler, Hunter, and Murphy (1935).

In a similar study Kline, Elvehjem, and Halpin (1935) conclude that the level of vitamin D in the hen's diet had little effect on the percentage of ash in the bones of the chicks. However, they emphasize that the results reported in their paper are for hens fed amounts of vitamin D which were within the range used under practical feeding conditions and that the feeding of much higher levels may have had an entirely different effect on the degree of bone calcification in the chick. In the work here reported, the hens of group 9 received approximately four times more vitamin D than is ordinarily used under practical conditions, while those in group 5 received an amount of vitamin D which Murphy, Hunter, and Knandel (1934) found to be optimum for laying hens denied access to sunlight.

SUMMARY

From the bone photographs and bone ash determinations of a total of 346 sets of bones evidence was secured to indicate that vitamin D is transmitted in a quantitative way from the hen to the chick. Evidence to show the vitamin D reserve of the chick is dependent upon the vitamin D intake of the parent stock can be secured when the chicks are from one to four weeks old.

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THE EFFECT OF THE VITAMIN A (CAROTENE) INTAKE ON THE VITAMIN D REQUIREMENT OF RATS IN THE PRODUCTION AND CURE OF RICKETS [1]

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(Received for publication April 1, 1936)

A vast amount of information regarding those factors which influence the physiological processes of ossification has accumulated during the past few years. Attention has been focused primarily upon the calcium and phosphorus content of the diet and upon the presence of the antirachitic substance or substances. It has been conclusively demonstrated that certain variations among these three factors will affect the structure of bone. Little appears to be known concerning the influence of other dietary factors upon the development and cure of rickets, although there are some indications that factors other than those mentioned above may be important in this connection. This is especially true of the vitamin A content of the diet. In fact, the relationship of this dietary factor to the incidence of rickets has already attracted the attention of a few investigators. Rohmer and Dubois ('30) on conducting a series of experiments upon rachitic children receiving a minimum effective dose of vitamin D (300 to 600 rat units of viosterol per day) accompanied by daily administration of 3000 rat units of vitamin A found that such a treatment did not modify the action of viosterol in any way. Bacharach, Allchorne and Hazley ('31) studied the effect of adding fresh carrot to a rachitogenic diet and came to the conclusion that the addition of 1 gm. of fresh carrot daily to such a diet did not affect the rate of growth of rats, whether given in the absence or in the presence of vitamin D. Furthermore, these investigators were unable to find any correlation between the severity of rickets and the rate of growth.

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[2] Presented by Florence S. Tabor to the faculty of the Department of Agricultural and Biological Chemistry of the Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Our knowledge of the marked physiological responses produced by vitamin A rich foods led us to believe that vitamin A might have some effect on the vitamin D utilization. Variations in the carotene content of yellow corn as well as in the Ca/P ratio may play a part in the variability of results which have been obtained in different laboratories. Since pure carotene is now available as a source of vitamin A and since viosterol is available as an uncontaminated source of vitamin D, it appeared desirable to study the problem further. Hence the experiments reported in this investigation were carried out for the purpose of determining whether a rachitogenic diet supplemented with different quantities of carotene, as a source of vitamin A, exhibited any influence upon the incidence and cure of rickets. The results obtained were evaluated from both the chemical and osteological findings, with attempts to correlate chemical analyses with radiographic and osteological data.

EXPERIMENTAL

In order to study the problem in question young growing animals of known ancestry were depleted of their body stores of vitamin D by feeding a rachitogenic diet and also by feeding a similar diet after it had been supplemented with different levels of carotene. At the end of the depletion period (twenty-first or twenty-third days) all diets were supplemented with equivalent amounts of vitamin D.

The basal diet used in these studies was the Steenbock yellow corn ration no. 2965 ('25) which consisted (in parts per 100) of yellow corn 76, wheat gluten 20, calcium carbonate 3 and sodium chloride 1. Since it was desirable to maintain a definite dietary composition in order to produce a comparable degree of rickets at all times during the course of the investigation, representative samples of the basal rachitogenic ration were analyzed and found to have the following composition (in parts per 100), protein 24.26, calcium 1.52, phosphorus 0.38.

The various supplements added to the basal diet were: a) Wesson oil, b) a carotene preparation,[3] containing 4200 U. S. P. units[4] of vitamin A per gram, c) a solution of viosterol in olive oil, containing 1 U. S. P. unit of vitamin D per milligram, and d) U. S. P. reference cod liver oil, containing 95 U. S. P. units of vitamin D per gram. An attempt was also made to use a fish-oil concentrate[5] which contained 2,025,000 U. S. P. units of vitamin A per gram as an added source of this vitamin in these studies. The vitamin D content of this product, however, was so great that severe rickets could not be obtained when the desired levels were fed.

Rapidly growing rats 20 to 23 days old and weighing from 39 to 45 gm. each were placed and maintained in individual metal cages, which were provided with raised screen grids. Care was taken to insure uniform distribution of litters and sexes throughout the various groups of experimental animals. During the entire experimental period all animals were kept in a darkened room which was ventilated by means of an electric fan. Distilled water was kept before the animals at all times. All animals were weighed at weekly intervals during the depletion period and a systematic record was made of all changes in body weight and food consumption. For the purpose of dietary treatment, these animals were arranged in experimental groups of eight to sixteen animals per group.

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[3] Acknowledgment is gratefully made to the S. M. A. Corporation of Cleveland for the Primatene used in this investigation.

[4] Guaranteed to contain not less than 3000 A. D. M. A. units per gram and converted to U. S. P. units by using conversion factor 1.4.

[5] Acknowledgment is gratefully made to Mr. Harden Taylor of the Atlantic Coast Fisheries, through whose courtesy a fish-oil concentrate of a very high vitamin A potency was obtained. According to Mr. Taylor this product was prepared from a halibut liver oil concentrate by molecular distillation. Previous tests by Dr. R. J. McWalter, University College, London, with the Hilger quartz echelon cell spectrophotometer gave a coefficient of absorption E of 960 at 3280A° through 1 cm. of 1 % solution. The exceedingly high potency ascribed to this product was calculated from data obtained under similar conditions on a concentrate prepared from a sample of U. S. P. reference cod liver oil (3000 units per gram).

At the end of the depletion period and again at the end of the curative period all animals were x-rayed to determine the state of calcification. At the latter date the animals from each group were sacrificed, the blood was analyzed for calcium and phosphorus, and the long bones of the leg were used for line testing and for bone ash analyses. In the case of the blood it was necessary to pool samples from several animals of the same group, otherwise sufficient amounts were not available for analysis. Duplicate determinations were made in all cases.

To compensate for the lack of sensitivity of the radiographic examination, representative animals from the different experimental groups were killed by bleeding, as controls at various stages of the depletion period, and line tests and bone ash determinations were made of all bones obtained from such animals. In addition, the blood of these animals was used for the determination of calcium and phosphorus.

At the end of the various depletion periods, the remaining animals in all groups were continued on the respective basal diets and, in addition, were given equal amounts of vitamin D of a definite unitage. After these animals had been carried through the desired experimental period, they were killed as described above. The degree of healing or calcium deposition was measured by the x-ray and line test methods and chemical determinations were made for bone ash, blood calcium and blood phosphorus.

The right and left femora and the tibiae and fibulae were used for the line test and bone ash determinations, respectively. These bones were dissected from the animals and freed from all adhering tissue immediately after the animals were killed. The femora were removed for line tests and photographic records, while the tibiae and fibulae were prepared for ashing by the usual extraction methods, care being exercised to prepare the bones in a uniform manner.

The proximal ends of the femora were examined at the termination of the curative period by the line test technic for evidence of recalcification. The tibiae and fibulae from each leg were wrapped in cheese cloth, dried for 24 hours in an electric oven at a temperature of 100°C., extracted continuously with hot alcohol for 8 hours, and again with ether for the same length of time. The bones were again dried to a constant weight as described above, were weighed in tared crucibles, placed in a muffle furnace and ashed for 4 hours at dull red heat. Crucibles and contents were cooled in a desiccator over night and weighed the following day. The percentage of bone ash was calculated on the basis of the dry extracted bone. Standard deviations for bone ash data were calculated.

Blood samples obtained by cardiac punctures were allowed to stand in the refrigerator over night in order that the serum might exude. The serum was pipetted off and centrifuged to free it from blood cells. An aliquot of the serum was analyzed for calcium by the Clark-Collip ('25) modification of the Kramer-Tisdall ('21) method.[6] The inorganic phosphorus content of the serum was determined by the Youngburg et al. method ('30).

DATA

An outline of the general plan of the investigation is given in table 1. In order to conserve space, summaries of data have been condensed to tabular form and are presented in tables 2 and 3.

The general plan, which was applicable to all groups in each series of experiments, consisted first in developing definite experimental rickets in animals under uniform experimental conditions by: a) Feeding rachitogenic diets to control groups, b) supplementing the basal rachitogenic diet

[6] The mixture was allowed to stand over night for complete precipitation of the calcium oxalate.

with definite quantities of carotene as the source of vitamin A, and c) supplementing the basal rachitogenic diet as well as the diets containing the added amounts of carotene with 5 % of Wesson oil.

It will be observed that the outline presented in table 1 has been divided into two parts, i.e., the depletion period and the curative period. It will also be observed that two sources of vitamin D were used as supplements during the curative

TABLE 1

General outline of the experimental procedure during depletion and curative periods

SERIES	GROUP NO.	DEPLETION PERIOD				CURATIVE PERIOD		
		Number of rats	Days on depletion	Supplements to basal rachitogenic diet		Number of rats	Days on curative	Total Vitamin D intake
				Vitamin A [1]	Wesson oil			
				U.S.P. units	%			*U.S.P. units*
A	1	4	21	..	..	8	18	18[2]
	2	4	21	22	..	8	18	18[2]
	3	4	21	90	..	8	18	18[2]
B	4	5	23	..	..	5	14	14[2]
	5	5	23	22	..	5	14	14[2]
	6	5	23	90	..	5	14	14[2]
C	7	5	23	..	..	5	12	12[2]
	8	5	23	22	..	5	12	12[2]
	9	5	23	90	..	4	12	12[2]
D	10	2	23	..	..	5	14	1[3]
	11	2	23	..	5	7	14	1[3]
	12	2	23	22	5	8	14	1[3]
	13	2	23	90	5	5	14	1[3]
E	14	4	21	..	..	7	14	3.99[4]
	15	4	21	..	5	8	14	3.99[4]
	16	4	21	22	..	8	14	3.99[4]
	17	4	21	22	5	8	14	3.99[4]
	18	4	21	90	..	7	14	3.99[4]
	19	4	21	90	5	8	14	3.99[4]
F	20	6	23	..	5	8	14	3.99[4]
	21	8	23	90	..	8	14	3.99[4]
	22	7	23	90	5	6	14	3.99[4]
G	23	6	21	..	..	11	16	...

[1] U. S. P. units for each gram of diet.

[2] Viosterol in petroleum ether.

[3] Viosterol in Wesson oil.

[4] U. S. P. reference cod liver oil in Wesson oil.

TABLE 2

Summary of the results obtained during the depletion period[1]

SERIES	GROUP NO.	SUPPLEMENTS TO BASAL RACHITOGENIC DIET		AVERAGE WEIGHT		AVERAGE DAILY FOOD INTAKE	AVERAGE DAILY VITAMIN A INTAKE	SERUM MILLIGRAMS PER 100 CC.		AVERAGE PER CENT BONE ASH	STANDARD DEVIATION
		Vitamin A [2]	Wesson oil	Initial	Final			Ca	P		
		U.S.P. units	%	*gm.*	*gm.*	*gm.*	*U.S.P. units*				
A	1	..	..	38.7	62.2	6.1	...	..	5.1	43.38	±0.79
B	4	..	..	42.4	63.6	5.0	...	6.8	..	33.59	±3.02
C	7	..	..	41.2	57.4	4.5	...	..	3.7	33.61	±1.21
D	10	..	..	34.5	55.0	5.5	...	..	Lost	25.80	±1.32
E	14	..	..	55.2	72.7	6.5	...	6.4	5.0	41.07	±2.59
D	11	..	5	44.5	61.0	5.5	...	..	4.8	32.48	±1.47
E	15	..	5	55.7	65.6	5.4	...	6.7	5.7	51.23	±0.23
F	20	..	5	57.6	66.6	5.7	...	6.0	4.8	41.97	±1.88
A	2	22	..	39.7	60.2	5.5	121	..	5.8	44.60	±1.17
B	5	22	..	39.2	61.2	4.9	108	6.2	..	31.14	±1.17
C	8	22	..	41.2	56.4	4.3	95	..	4.1	32.47	±2.43
E	16	22	..	48.0	66.0	5.2	114	5.2	5.8	48.34	±1.42
D	12	22	5	42.0	52.0	4.9	108	..	Lost	30.55	±3.45
E	17	22	5	45.0	63.0	4.8	106	6.8	6.1	43.35	±0.59
A	3	90	..	42.7	61.7	4.3	387	..	4.1	38.76	±0.42
B	6	90	..	44.8	68.8	5.5	495	7.0	..	30.87	±1.44
C	9	90	..	40.4	57.2	5.6	504	..	4.0	32.20	±2.17
E	18	90	..	43.0	61.0	5.2	468	5.9	5.6	43.22	±1.93
F	21	90	..	60.0	77.5	5.9	531	5.6	4.1	42.81	±3.33
D	13	90	5	40.5	54.5	5.0	450	..	5.2	29.30	±3.95
E	19	90	5	48.8	62.5	5.4	486	6.2	5.2	42.61	±1.06
F	22	90	5	54.3	63.9	5.1	459	4.8	5.2	44.04	±3.06
G	23	..	..	37.8	59.6	4.9	...	6.8	..	32.24	±2.88

[1] For number of rats used and length of depletion period see table 1.

[2] U. S. P. units for each gram of diet.

TABLE 3

Summary of the results obtained during the curative period[1]

SERIES	GROUP NO.	SUPPLEMENTS TO BASAL RACHITOGENIC DIET		TOTAL VITAMIN D INTAKE	AVERAGE WEIGHT		AVERAGE DAILY FOOD INTAKE	AVERAGE DAILY VITAMIN A INTAKE	LINE TEST AVERAGE	SERUM MILLIGRAMS PER 100 CC.		AVERAGE PER CENT BONE ASH	STANDARD DEVIATION
		Vitamin A [2]	Wesson oil		Initial	Final				Ca	P		
		U.S.P. units	%	*U.S.P. units*	*gm.*	*gm.*	*gm.*	*U.S.P. units*					
A	1	..	..	18[3]	66.1	74.5	5.2	...	2.5	...	4.2	48.96	±0.31
B	4	..	..	14[3]	59.4	63.8	4.8	...	3.8	6.2	..	32.59	±1.26
C	7	..	..	12[3]	55.0	59.6	4.5	...	2.8	...	4.9	33.97	±2.83
D	10	..	..	1[4]	54.6	49.6	4.0	...	1.0	...	5.2	24.18	±5.01
E	14	..	..	3.99[5]	66.5	66.0	5.0	...	1.0	11.3	6.9	36.36	±3.43
D	11	..	5	1[4]	62.0	68.5	6.1	...	0.7	...	5.9	32.62	±5.87
E	15	..	5	3.99[5]	57.7	58.3	3.9	...	1.3	11.4	6.0	45.63	±1.36
F	20	..	5	3.99[5]	67.3	74.0	6.0	...	1.5	11.4	5.1	43.97	±8.53
A	2	22	..	18[3]	58.5	68.2	4.3	95	1.7	...	3.5	47.96	±1.84
B	5	22	..	14[3]	66.8	73.5	4.9	108	2.5	Lost	..	35.42	±1.40
C	8	22	..	12[3]	56.2	61.8	4.2	92	2.2	...	5.0	33.02	±3.71
E	16	22	..	3.99[5]	67.1	68.1	5.5	121	1.6	10.9	5.3	44.67	±1.59
D	12	22	5	1[4]	65.0	70.8	5.3	117	0.9	...	7.0	32.86	±7.25
E	17	22	5	3.99[5]	64.6	64.6	3.9	86	1.0	11.5	5.6	46.09	±2.37
A	3	90	..	18[3]	60.4	68.5	4.4	396	2.1	...	5.1	39.63	±1.72
B	6	90	..	14[3]	63.8	67.8	4.6	414	2.6	7.0	..	34.03	±4.17
C	9	90	..	12[3]	57.5	64.7	4.5	405	1.5	...	6.1	32.29	±1.85
E	18	90	..	3.99[5]	71.0	76.4	5.6	504	0.7	9.5	6.8	48.34	±3.30
F	21	90	..	3.99[5]	70.4	81.6	6.7	603	2.3	7.4	5.6	42.78	±3.33
D	13	90	5	1[4]	54.8	58.4	3.4	306	0.6	...	6.9	27.65	±8.27
E	19	90	5	3.99[5]	58.5	62.0	4.1	369	0.8	6.9	5.3	42.11	±2.45
F	22	90	5	3.99[5]	68.0	76.1	5.0	450	0.9	6.1	6.1	44.47	±2.40
G	23	..	..	...	41.2	68.3	5.4	...	..	10.3	3.8	27.75	±2.28

[1] For number of rats used and length of curative period see table 1.

[2] U. S. P. units for each gram of diet.

[3] Viosterol in petroleum ether.

[4] Viosterol in Wesson oil.

[5] U. S. P. reference cod liver oil in Wesson oil.

periods; namely, irradiated ergosterol in olive oil and U. S. P. reference cod liver oil. Either Wesson oil solutions or petroleum ether solutions of the vitamin D supplements were used. The data have been likewise summarized with reference to depletion or curative periods and are presented in tables 2 and 3, respectively.

The values for serum calcium and serum phosphorus as given in tables 2 and 3 represent group averages. Owing to limited supplies of blood serum from certain groups of animals, it was not feasible to determine both calcium and phosphorus. Degrees of healing are expressed by line test values in the conventional manner, i.e. [(—) (±) (+) (++) (+++)]. The degree of recalcification which is characterized by an unbroken thin line of darkened granules appearing on the metaphyseal side of the epiphyseal cartilage is expressed by (++), greater or lesser degrees of recalcification being designated accordingly.

In the first series of experiments (series A), three groups of animals (twelve animals per group) were used. The animals of group 1 received the unsupplemented Steenbock rachitogenic diet. The animals of group 2 received the above diet after sufficient carotene (see footnote 3) had been added to furnish 22 U. S. P. units of vitamin A for each gram of food, while the animals of group 3 received a similar diet fortified with carotene to the extent of 90 U. S. P. units of added vitamin A for each gram of food. The diets used in each series of experiments of this type were made up in quantities sufficient to complete the experiments, stored in air-tight containers, and maintained at a low temperature in an electric refrigerator. After the animals comprising this series had been on the rachitogenic diets for 21 days, four animals from each group were killed and used as controls. The remaining eight animals of each group received, in addition to the respective basal diets, 1 U. S. P. unit of vitamin D per day in the form of viosterol. The feeding of this vitamin D supplement was continued for a period of 18 days, at the end of which time the animals were killed, line tested, etc.

The animals in the second and third series of experiments (B and C) were depleted for a period of 23 days, since it appeared highly desirable to obtain a more marked degree of rickets than had been observed in series A. The curative periods were decreased to 14 and 12 days, respectively, since, in the first series (series A), recalcification was so advanced in all groups that sharp differentiation between groups was difficult.

Further variations were introduced in series D, through the introduction of a definite amount of fat in each of the above diets. In this series four groups of animals were used. The animals of group 10 received the usual rachitogenic diet, while group 11 received this basal diet after 5 % of Wesson oil had been added to it. Groups 12 and 13 received the diet containing 5 % of Wesson oil, which also contained 22 and 90 units of vitamin A per gram, respectively. The vitamin D supplement used in this series was viosterol which was made up to such a volume (in Wesson oil) that 1 cc. was equivalent to 1 U. S. P. unit. This supplement was fed in special receptacles on the first, fifth and tenth days in 0.4 cc., 0.3 cc. and 0.3 cc. portions.

In order to study the effect of the presence of fat in the diet on the vitamin A-vitamin D relationship, a fifth series of experiments was carried out. This series of experiments (series E) consisted of six groups of animals. In the beginning each group was composed of twelve animals, but unfortunately a number of these animals died during the course of the curative period. The depletion period for this series was 21 days, since radiographic examination at that time revealed that all animals showed a marked degree of rickets. The animals of this series were arranged in groups in such a manner as to make possible a comparison of the responses of those receiving diets supplemented with 5 % of Wesson oil with the responses made by other groups receiving the rachitogenic diet containing no added fat. The vitamin D supplement for this series was a solution of U. S. P. reference cod liver oil containing 95 U. S. P. units of vitamin D per gram. This vitamin D supplement was fed in separate containers in 0.5 cc. portions on the first, fifth and tenth days. This amount of oil was equivalent to 3.99 U. S. P. units.

Series F constituted a repetition of a portion of the feeding trials of series E, i.e., of groups 15, 18 and 19. In this series the depletion period was extended to 23 days because x-ray examinations on the twenty-first day revealed that the degree of rickets manifested at that time was somewhat less than was desirable.

The animals comprising series G were used as negative controls. These animals were maintained for 37 days on the basal rachitogenic diet without additional supplement. At the end of this period, the animals were examined to determine the state of ossification, using the various criteria described elsewhere.

DISCUSSION

These experiments were designed primarily for the purpose of determining whether vitamin A exerts any influence upon vitamin D utilization by the rat, the vitamin A being fed at different levels, while the amount of vitamin D remained constant. While agreement of opinion regarding the cause and cure of rickets is still lacking, four methods of evaluating the results in the incidence and cure of this disorder have been quite well established; namely, x-ray, line test, bone ash and chemical analyses of the blood for inorganic constituents, especially calcium and phosphorus. These criteria have been used in an attempt to establish the etiological interdependence of vitamins A and D in the production and cure of rickets. In many instances lack of correlation between these criteria has been strikingly demonstrated.

The effect of adding carotene as the source of vitamin A to the basal rachitogenic diet may be observed from the results obtained in series A (table 2). Mild rickets was manifested by animals comprising the control group (group 1) as well as by those animals of group 2 which received the rachitogenic diet supplemented by 22 units of vitamin A.

Severe rickets was very apparent among those animals of group 3 which received the basal rachitogenic diet supplemented by 90 units of vitamin A. Similar observations were made among comparable groups of animals in series B and C. The marked similarity of results obtained from the several groups of animals comprising series D indicates that when Wesson oil was added to the diet there was no appreciable effect on the severity of rickets produced, in spite of the fact that the total gain in weight made by the animals on the low fat diet (group 10) exceeded that of all other groups of the series. A discrepancy appears in the results obtained from all groups of series E. Rickets was evident among the animals of the control group (group 14), while no indication of this disorder appeared in group 15 and only very mild rickets was observed in groups 16, 17, 18 and 19 of this series. Examination of the data does not disclose that the animals had either lost weight or were refusing food but, on the contrary, all groups had shown appreciable gain in body weight. In order to verify the results obtained in the above series, this series (series E) was repeated using three groups of animals (series F). But here again the animals of the control group (group 20) showed evidence of definite rickets, whereas those of groups 21 and 22 manifested no greater severity than had previously been shown by comparable groups in the preceding series.

In these studies x-ray technic was found to be less accurate than the osteological technic in determining the degree of rickets. It was found, however, to be sufficiently accurate to indicate that the majority of animals receiving diets supplemented with 90 units of vitamin A per gram of food manifested a more marked degree of rickets than did the control animals or those animals receiving lower levels of vitamin A.

A second criterion for estimating the intensity of rickets was the variation in bone ash values. An inspection of these data shows a serious lack of consistency. In the case of series A high bone ash values were obtained for the animals of groups 1 and 2 (43.38 and 44.60 %) and decidedly lower figures were obtained for those of group 3 (38.76 %). Similar irregularities, although less marked, were obtained in series B and C.

There appeared to be no appreciable differences in the average bone ash values among the groups of animals comprising these two series. The bone ash values obtained in series D were noticeably lower than those which had been obtained in any of the preceding experiments. It should be noted, however, that this constituted a preliminary series in which an insufficient number of animals was used. The bone ash value for the control group (group 10) was the lowest obtained (25.80 %), although it was noted that the animals in this group made a greater increase in body weight than did the animals of the succeeding groups. It is possible to explain the low figure mentioned above on the assumption that rate of growth is recognized as a predisposing factor in the etiology of rickets. The bone ash values for the animals of the remaining groups in the series were 32.48, 30.55 and 29.30 %, respectively.

Since the data obtained in the experiment of series D indicated some tendency toward differences in bone ash values when 5 % of Wesson oil was used as an added supplement to the rachitogenic diet, it seemed advisable to repeat the experiments of series D using a larger group of animals. The data obtained in this series (series E) disclose surprisingly inconsistent results. The animals of group 14 gave an average bone ash value of 41.07 %, yet x-ray examination had indicated severe rickets in all animals of the group. The animals in group 15 manifested little or no evidence of rickets and the average bone ash value for the group was 51.23 %. In group 16 two animals gave no indication of rickets when observed radiographically, two animals showed mild rickets, and the average bone ash value for all animals of this group was 48.34 %. The bone ash values obtained for groups 17, 18 and 19 were 43.35, 43.22 and 42.61 %, respectively.

In an attempt to clarify the discrepancies just described, another experiment consisting of three groups of animals was carried out. The three groups of animals composing this series (series F) received the same dietary treatment as those of groups 15, 18 and 19. The animals of group 20 of this series exhibited definite rickets in all cases, although the average bone ash value remained at 41.97 % or considerably lower than the value obtained in group 15 of the preceding series. Values for groups 21 and 22 were in agreement with those obtained in series E, in that there was no appreciable lowering of bone ash values although rickets was manifest to a certain degree in all animals of the series.

Examination of the data in table 2 discloses a wide variation in bone ash values. It is apparent that definite correlation does not always exist between severity of rickets (as measured by line tests) and bone ash determinations, although duplicate determinations, when made on the bones of individual animals, agree consistently. A factor which may have some bearing on the above discrepancies is the age of the test animals. Bone ash determinations on the extracted bones from normal animals of various ages gave the following values: 18 months, 63.20 % ± 1.16 (S.D.)[7]; 12 to 15 months old, 56.03 % ± 1.36 (S.D.); 40 to 50 days old, 50.35 % ± 2.01 (S.D.); and 30 to 40 days old, 48.34 % ± 1.64 (S.D.). The value obtained for negative controls, animals at the end of a 37-day depletion period, was 27.80 % ± 2.28 (S.D.).

Blood phosphorus values obtained in series A, C and D disclosed no significant differences. Blood calcium values obtained in series B were somewhat lower than normal values, although there was not a very great difference between individual groups of the series. The average value for the animals of the control group (group 4) was 6.8 mg. of calcium per 100 cc. of serum, while the values for the other two groups were 6.2 and 7.0 mg. per 100 cc. of serum respectively.

In the case of the animals comprising the various groups of series E and F, both blood calcium and blood phosphorus values were determined. An examination of the data in table 2 shows that the product of these values for all groups, except group 17, was 30 or less. Kramer, Shear and Siegel ('31) have reported that active rickets is present when this product falls below 30. The radiographic and bone ash values for these experiments, however, do not warrant such a conclusion. The addition of 22 and 90 units of vitamin A tended to lower blood calcium and blood phosphorus values, although the differences between groups were slight. Attention is called to the fact that bone ash values remained consistently high in spite of the appreciable lowering of blood calcium and blood phosphorus. In other words, there does not seem to be a definite correlation between bone ash, blood calcium and blood phosphorus values as obtained in these series of experiments during the depletion periods.

The influence of vitamin A on the vitamin D utilization and, consequently, on the cure of rickets may be deduced by likewise considering data obtained in the curative periods. An analysis of the line test data as summarized in table 3 shows that, in general, lower values for average line test healings were observed in all groups receiving vitamin A supplements than were observed among animals of the control group. The lowest values were observed among those groups receiving the basal ration supplemented with 90 units of vitamin A and 5 % of Wesson oil simultaneously. There appears to be less correlation between bone ash values and line test values at this time than there was between radiogram and bone ash values at the end of the depletion periods.

Further examination of the data discloses that wide variation in bone ash values occurred among the various groups of test animals. In series A there was no definite increase in bone ash in any of the groups over and above that observed at the close of the depletion period. Group 3, with the lowest value of 39.63 %, gave a relatively high line test average (2.1), while group 2 with a bone ash value of 47.96 % gave a line test average of only 1.7. More striking discrepancies appear among the bone ash data of series B and C; yet all of these groups gave high line test averages. Relatively low bone ash values were found for all groups of animals com-

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[7] S.D. (standard deviation).

prising series D. The lowest bone ash value was given by the control group (group 10); however, this group gave the highest line test value.

Inconsistencies in bone ash values are further emphasized in those groups comprising series E. The control group (group 14) gave a lower value for bone ash at the end of the curative period than was observed at the end of the depletion period. Similar observations are noted among the animals of groups 15 and 16. Groups 17 and 18 manifested a marked increase in bone ash values for the curative period, while group 19 showed little difference between bone ash values at the end of the depletion period and at the end of the curative period. The results obtained in series F were very comparable to those obtained in series E. On the basis of these bone ash data only indefinite information is afforded relative to the degree of recalcification. This opinion has been expressed by other investigators (Adams and McCollum, '28).

Since there appeared to be a definite lack of correlation between bone ash values and the corresponding line test averages, an attempt was made to determine the range in line test values which would result in a measurable difference in the percentage of bone ash. For this purpose the tibiae (fibulae attached) from both legs of four animals which had shown a 4(+) line test healing were removed and bone ash values were determined according to the technic previously described. The average bone ash value obtained for this group of animals was 38.5 % ± 3.6 (S.D.). This same procedure was repeated using six animals, which gave a 1(+) line test healing. The average bone ash value for this group was 33.1 % ± 2.47 (S.D.). In other words, a difference between a 4(+) and a 1(+) in line test values was necessary to show a difference of approximately 5 % in bone ash values. It appears, therefore, that small differences in the degree of recalcification cannot be measured quantitatively by the bone ash technic.

Variations in blood calcium and blood phosphorus were obtained among those series of animals which had received vitamin D from different sources as a supplement to the rachitogenic diets. One U. S. P. unit of vitamin D daily (viosterol in petroleum ether) was the supplement used in series A, B and C. In general, it may be pointed out that very small increases in the values over those found at the end of the depletion period were manifested. Blood phosphorus values were determined for series A and C. In series A this value increased in group 3, while for groups 1 and 2 the value was lower than at the end of the depletion period. In series C all groups manifested an increase in blood phosphorus; group 9 (receiving 90 units of vitamin A per gram of food intake) gave the highest value. Blood calcium was determined for series B. The increase in this element over that observed at the end of the depletion period was very slight. In series D the supplement was 1 U. S. P. unit of vitamin D (viosterol in Wesson oil). In this series an increase in the blood phosphorus was observed among all groups of animals, the values ranging from 5.2 to 7.0 mg. of phosphorus per 100 cc. of serum.

Blood calcium and blood phosphorus values were determined in series E and F, and here some interesting observations were made. While marked increase appeared in the calcium values above those observed at the end of the depletion periods, there was no great increase in the blood phosphorus values of either series. No difference in the blood calcium value for the control group (group 14) and the group which received the rachitogenic diet plus the Wesson oil (group 15) was apparent. A similar value (11.5 mg. of Ca per 100 cc. of serum) was given by group 17, while lower values were obtained in groups 16, 18 and 19. These values ranged from 10.9 to 6.9 mg. of Ca per 100 cc. of serum.

There appeared to be but slight differences in the blood phosphorus values among the various groups of these series, the values ranging from 6.9 to 5.3 mg. per 100 cc. of serum.

The values for series F were similar to those for corresponding groups in series E. In this series a reference cod liver oil, in an amount equivalent to 3.99 U. S. P. units of vitamin D, was fed as the supplement. The average blood calcium and blood phosphorus values for groups of control animals on this supplement after a 10-day curative period was found to give 11.4 and 5.2 mg. per 100 cc. of serum, respectively.

An examination of the values obtained by the chemical analyses of the blood serum shows a lack of correlation between calcium and phosphorus values, line test values, and bone ash values. Differences of opinion and conflicting results concerning such findings appear in the literature. It has been observed by Kramer, Shear and Siegel ('31) that recalcification in rachitic bones is preceded by a rise in the blood calcium and phosphorus. Hess et al. ('30) have observed that there is a lack of correlation between healing rickets and the inorganic phosphorus of the blood and that, while in general a lowering of phosphorus takes place, this is not necessarily an essential feature of rickets.

The age of the test animal may have some bearing on the diversity of results. Analyses of blood for inorganic calcium and phosphorus from normal animals of various ages gave the following values in milligrams per 100 cc. of serum: 3 to 5 weeks—calcium 7.6, phosphorus 8.7; 90 days—calcium 9.8, phosphorus 6.6; 17 months—calcium 9.6, phosphorus 6.3; 20 months—calcium 10.9, phosphorus 6.3.

SUMMARY

The production of rickets in the growing rat receiving a rachitogenic diet supplemented and unsupplemented with carotene as the source of vitamin A has been studied by means of x-ray examination, line test, bone ash determinations, and by an analysis of the blood serum for calcium and phosphorus. The subsequent recalcification, when corresponding animals received such diets supplemented with vitamin D from two sources, has been investigated in a similar manner.

The manifestations of the interrelationship of vitamin A and vitamin D in the etiology of rickets depend no doubt upon several factors, among which may be mentioned 1) past ancestral and nutritional history, 2) the age of the animals, and 3) individual variation among animals of the same age. It may be observed that the values found for the percentage of bone ash for normal animals of different ages varies with age. There is also variation in the blood calcium and blood phosphorus among normal animals of different ages. It is, therefore, highly desirable that all experimental animals used in a study of this nature be from the same genetic source and be carefully standardized according to age.

The efficient conversion of carotene to vitamin A undoubtedly varies among individual animals. Consequently, a detailed study of the problem necessitates the use of a natural vitamin A concentrate free from vitamin D in order to insure a clearer understanding of the vitamin A-vitamin D relationship. Until it is possible to procure such a supplement, this interrelationship cannot be readily demonstrated.

The following observations were made during the course of the experiment:

Rachitogenic diets containing 90 U. S. P. units of vitamin A per gram of diet seemed to produce, during the depletion period, an increase in the severity of rickets among growing rats over and above that produced by a similar diet which did not contain added vitamin A supplement.

The radiographic examination at the end of the depletion period was a more reliable index of the intensity of rickets than was the percentage of bone ash.

Evidences of satisfactory correlation between the percentage of bone ash and corresponding radiograms were not obtained.

Inconsistencies between the calcium and phosphorus content of the blood serum and bone ash values were also apparent.

When equivalent unitages of vitamin D were administered, rachitogenic diets containing 90 U. S. P. units of vitamin A per gram, with and without 5 % of Wesson oil, appeared to retard recalcification (as measured by line test technic) to a greater extent than was observed when similar diets which did not contain any vitamin A supplement were fed.

Data obtained as a result of bone ash determinations and blood serum analyses did not appear to be in agreement with line test data.

From the results obtained in this investigation the line test data appeared to be the most reliable criteria of recalcification.

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PRE-HARVEST SPRAYING OF APPLES FOR REMOVAL OF LEAD RESIDUES

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PRE-HARVEST SPRAYING OF APPLES FOR REMOVAL OF LEAD RESIDUES[1]

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The advantages of a wash solution that could be applied to fruit hanging on the trees to reduce the level of spray residues are obvious. Growers already possess the machinery necessary for application. In orchards where residues at harvest customarily exceed the tolerance by a relatively slight margin, such treatment might obviate the necessity for special washing treatment before packing. Where almost continuous spraying produces residues that now require drastic and expensive washing treatment, a successful pre-harvest residue-removing application might permit a milder washing treatment to produce satisfactory results.

During the season of 1935, 18-year old trees of the Stayman Winesap and York Imperial varieties were given cover sprays, as indicated at the top of table 1, on June 18 and 25, July 2 and 16, and August 12. Other Stayman Winesap trees that had received a fourth cover spray early in July were given a final application of oil–lead in mid-August. For a preliminary study of residue-removing sprays, three materials were applied to the trees with a power sprayer on September 15. These materials were hydrated lime 10 pounds per 100 gallons, ammonium nitrate 8 pounds per 100 gallons, and paste *Vatsol* one-half pound per 100 gallons, each at the rate of 10 gallons per tree. A greater concentration of *Vatsol* might have been desirable, but greater amounts produced excessive foaming under agitation in the spray tank.

Duplicate fruit samples for analysis were picked from the lower limbs of the trees, and duplicate determinations for lead were made by the method of Frear & Haley.[2] Samples were taken on September 9, before the sprays were applied, and at harvest on October 15. Further samples taken in an identical manner at harvest were given a one-minute exposure to 1.5 per cent hydrochloric acid in a flotation washer before analyzing for lead.

The complete data for the experiment are presented in table 1. Doubtless because the figure given for each combination involves fruit samples from a single tree only, the data show considerable variation. For this reason no attempt will be made to distinguish between the two control schedules employed on both varieties. However, with one exception (following supplementary treatment with ammonium nitrate) the averages show a residue more difficult to remove from York Imperial than from Stayman Winesap, a result in line with previous apple-washing experience. For this reason, comparisons of the average effectiveness of the three residue-removing sprays tested may be justified.

Two questions present themselves. First, is the natural loss of lead per

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[1] Authorized for publication on December 17, 1935, as paper No. 716 in the Journal series of the Pennsylvania Agricultural Experiment Station.

[2] **Frear, D. E. H.** and D. E. Haley, 1934. A simplified method for the rapid determination of lead residues o apples. Pa. Ag. Exp. Sta. Bul. **304**.

Table 1.—Lead residues on apples as affected by supplementary sprays applied on September 15. The amounts are in grains per pound.

Sample Taken	Date	Five Cover Sprays of Lead Arsenate 3:100 plus							Orthol K 1:100 in Last Cover Only
		Lime Sulfur 1:50		Flotation Sulfur 5:100 plus Fish Oil 1:400		Average			
		Stayman	*York*	*Stayman*	*York*	*Stayman*	*York*	*Total*	*Stayman*
Before supplementary treatment	Sept. 9	.109	.098	.107	.098	.108	.098	.103	.089
After supplementary spray of hydrated lime 10:100									
At harvest	Oct. 15	.065	.068	.059	.063	.062	.066	.064	
After washing	Oct. 17	.011	.011	.007	.008	.009	.01	.009	
After supplementary spray of ammonium nitrate 8:100									
At harvest	Oct. 15	.047	.071	.079	.039	.063	.055	.059	.068
After washing	Oct. 17	.009	.012	.012	.007	.011	.01	.01	.008
After supplementary spray of paste *Vatsol* .5:100									
At harvest	Oct. 15	.071	.049	.045	.074	.058	.062	.06	.062
After washing	Oct. 17	.009	.015	.009	.009	.009	.012	.011	.008
After no supplementary treatment									
At harvest	Oct. 15	.073	.081	.049	.057	.061	.069	.065	.076
After washing	Oct. 17	.012	.019	.011	.008	.012	.014	.013	.01
Average									
At harvest	Oct. 15	.064	.067	.058	.058	.061	.063	.062	.069
After washing	Oct. 17	.01	.014	.01	.008	.01	.012	.011	.009

Table 2.—Percentage of reduction in lead residues, September 9 to October 15.

	Supplementary Treatment			
	None	Lime	Ammonium Nitrate	*Vatsol*
Average Stayman	43.5	42.6	41.7	46.3
Average York	29.6	32.6	43.9	36.7
Average total	36.9	37.8	42.7	41.7
Increase over check		.9	5.8	4.8
Oil-sprayed Stayman	14.6		23.6	30.3
Increase over check			9	15.7

Table 3.—Percentage of lead residue removed by washing, one-minute exposure to 1.5 per cent HCl.

	Supplementary Treatment			
	None	Lime	Ammonium Nitrate	*Vatsol*
Average Stayman	80.3	85.5	82.5	84.1
Average York	79.7	84.8	81.8	80.7
Average total	80	85.1	82.2	82.5
Increase over check		5.1	2.2	2.5
Oil-sprayed Stayman	86.8		88.2	87.1
Increase over check			1.4	.3

unit of weight while the fruit remains on the trees accelerated by the supplementary treatment? Second, does the use of such a supplementary spray treatment facilitate cleaning after harvest? Table 2, which has been calculated from the figures in table 1, reveals the extent to which the lead residues present on September 9 had disappeared by October 15 due to the growth of the fruit, to weathering and to the supplementary spray treatments of September 15. Because under the conditions of the experiment the first two of these factors exerted a uniform effect, differences may be attributed to the residue-removing sprays.

None of the materials used appeared to increase the loss of lead to any significant extent except from the Stayman Winesap sprayed with lead arsenate plus mineral-oil emulsion. On this fruit *Vatsol* showed considerable benefit in a single test, though under the conditions of the experiment the residues remaining at harvest were far above the legal tolerance. During the period from September 9 to October 15 precipitation totaled 1.66 inches, or about half the normal rainfall for this period. Earlier application of the supplementary sprays, and a subsequent heavy drenching with clear water, particularly if the weather remained dry, might increase the residue-removing effect of these materials.

Table 3 has been calculated in a manner similar to table 2, and shows the extent to which the uniform post-harvest acid wash removed the residues remaining at harvest in the various treatments.

With the possible exception of lime, the figures in table 3 indicate that the supplementary sprays exerted little beneficial effect on the removal of the residue by hydrochloric acid in a flotation washer.

Several generalizations are possible from tables 2 and 3. It was necessary to wash all of the fruit with hydrochloric acid to reduce the residue below the legal tolerance. The effect of this acid wash was to reduce the residues on all treatments to nearly the same level; hence, in the end little practical benefit may be ascribed to any of the supplementary treatments under the conditions of this test. The figures presented in table 2 indicate that under certain conditions some of the supplementary treatments assisted the natural processes of growth and weathering in reducing the residues on the fruit at harvest; with the materials and methods used this benefit was slight. However, since in many Pennsylvania orchards residues at harvest usually exceed the legal tolerance by comparatively slight margins, it is felt that continuation of these studies is justified.

The Illinois Natural History Survey and the Illinois Agricultural Experiment Station, Urbana, have entered into a cooperative agreement with the federal Bureau of Entomology and Plant Quarantine for studies of the corn ear worm. R. A. Blanchard of the Bureau has established headquarters at Urbana and A. F. Satterthwait will join him soon. The objective of the studies is development of better and more practical measures for control of corn ear worm.

E. O. Essig, University of California, Berkeley, will be on sabbatical leave from July 1, 1936, to June 30, 1937. From July 1 to September 1, 1936, and from March 1 to June 1, 1937, his address will be Cambridge University, Cambridge, England. During the period from September 1, 1936, to March 1, 1937, his address will be the National Museum du Congo, Tervueren, Belgium.

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BULLETIN 334 AUGUST, 1936

The Vitamin D Requirements of Growing Chicks and Laying Hens

age Yearly Egg Production of Pullets Which Received Adequate Vitamin D (167 Eggs)

Average Yearly Egg Production of Pullets Which Received Inadequate Vitamin D (79 Eggs)

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
STATE COLLEGE, PENNSYLVANIA

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The Vitamin D Requirements of Growing Chicks and Laying Hens[1, 2]

R. R. MURPHY, J. E. HUNTER, AND H. C. KNANDEL

WITHIN the past few years, poultry management practices have changed to such an extent, that chicks are now hatched at any season of the year. Late fall and early spring hatching has made it necessary to confine the chicks for most of the rearing period. When the range is very limited or the soil contaminated it is desirable to keep the fowls confined throughout the growing and laying periods. In order that chicks may be reared successfully at any season of the year one of the essential requirements is a sufficient amount of vitamin D. For satisfactory results with confined laying hens it is necessary to supply ample amounts of vitamin D.

Most of the investigations dealing with the vitamin D requirements of poultry have related only to the growing period. The work here reported, which covered a period of four years, is a study of the vitamin D requirements of growing chicks, laying fowls, breeding hens, and the effect of various levels of vitamin D fed to the mother hen on the resultant offspring.

The investigation consisted of two series of experiments. The former was conducted during the years 1932 and 1933 and the latter during the years 1934 and 1935. Complete results of the 1932-33 study were published by the Pennsylvania Agricultural Experiment Station in Bulletin 303, "The Vitamin D Requirements of Growing Chicks and Laying Hens." In the present publication these results have been combined with those of the 1934-35 experiment.

The product used was a cod liver oil vitamin D concentrate in cod liver oil[3] manufactured by the donor of this fellowship. This product is hereinafter referred to as cod liver oil concentrate. It was assayed with both chicks and rats to determine its vitamin D potency. Three independent laboratories co-operated in testing this oil. By the rat assay method (U.S.P.X. Revised 1934) the cod liver oil concentrate used in these experiments was found to contain 310 U.S.P. units of vitamin D per gram. In Bulletin 303, Pennsylvania Agricultural Experiment Station, the potency of the concentrate used was given as 100 Steenbock units or 270 U.S.P. units of vitamin D per gram. At the time that work was done the Steenbock method of assay was used for determining the potency of cod liver oil and a conversion factor of 2.7 used for expressing the unitage in U.S.P. units. Since some of the results reported in Bulletin 303 are also included in this publication they have been recalculated using the potency of the cod liver oil concentrate as 310 U.S.P. units of vitamin D per gram.

Experimental Procedure, 1932-33 Experiment

Ten groups each consisting of 200 Single Comb White Leghorn chicks of the Pennsylvania State College strain were hatched and placed on experiment April 12, 1932. Each chick was individually weighed and wing-banded at

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[1] Publication authorized June 1, 1936.

[2] This work was made possible through the establishment of a fellowship by the National Oil Products Company, Harrison, New Jersey.

[3] Reinforced in vitamin D by the Theodore F. Zucker process, United States patent number 1,678,454.

hatching time. These chicks were subjected to the following experimental conditions.

Group number	*Environmental conditions 0 to 12 weeks*	*Per cent cod liver oil concentrate in total ration*	*U.S.P. units vitamin D per 100 grams feed*
1	Battery brooder-confined	None	0
2	" " "	1/32	10
3	" " "	1/16	19
4	" " "	1/8	39
5	" " "	3/16	58
6	" " "	1/4	78
7	" " "	3/8	116
8	" " "	1/2	155
9	Colony brooder house-limited range	None	0
10	Colony brooder house-limited range	1/8	39

Groups 1 to 8 were brooded in batteries, each deck of which was heated indirectly by hot water so that all decks could be maintained at a uniform temperature. The brooders were located in a well ventilated room equipped with auxiliary heat. No direct rays of the sun were allowed to enter the room.

Groups 9 and 10 were brooded in colony brooder houses of similar size and construction, and heated with hard coal-burning brooder stoves. Limited range was provided for these two groups throughout the entire year in order that the chicks might be exposed to direct sunlight. All groups (1 to 10 inclusive) were fed the following all-mash rations:

	Starting mash *	*Growing and laying mash* †
	Pounds	*Pounds*
Ground yellow corn	43.5	45.5
Wheat bran	10.0	10.0
Standard Wheat middlings	10.0	10.0
Alfalfa meal	5.0	5.0
Meat scraps (55% protein)	10.0	7.5
Dried skim milk	10.0	7.5
Ground heavy oats	10.0	10.0
Ground limestone (98% $CaCO_3$)	1.0	4.0
Salt	0.5	0.5
Total	100.0	100.0
Per cent protein	18.40	16.40
Per cent calcium	1.47	2.79
Per cent phosphorus	0.92	0.80

* Fed from one-day old to 12 weeks.
† Fed from 12 to 76 weeks of age

When four weeks old, cockerels from all groups were removed. At 12 weeks of age, 65 representative pullets from each of the 10 groups were transferred to the laying pens. Body weight, health, condition, physical development, and state of maturity were considered in making the selection. At this time the growing and laying mash was subsituted for the starting mash. The 10 groups of pullets were housed in a long continuous type of laying house. Direct sun-

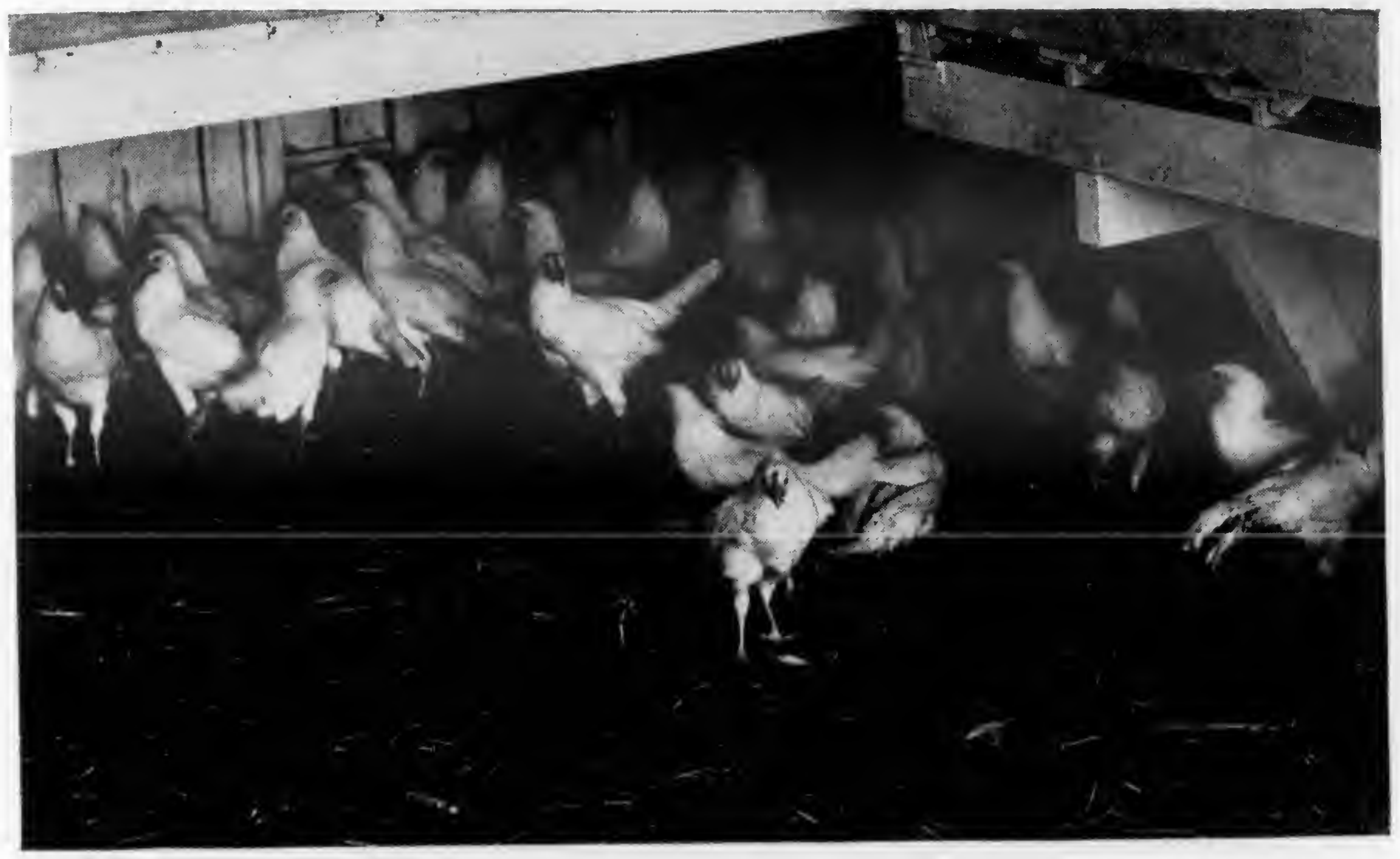


FIG. 1.—EXPERIMENTAL PULLETS, 16 WEEKS OLD, WHICH RECEIVED INADEQUATE AMOUNTS OF VITAMIN D

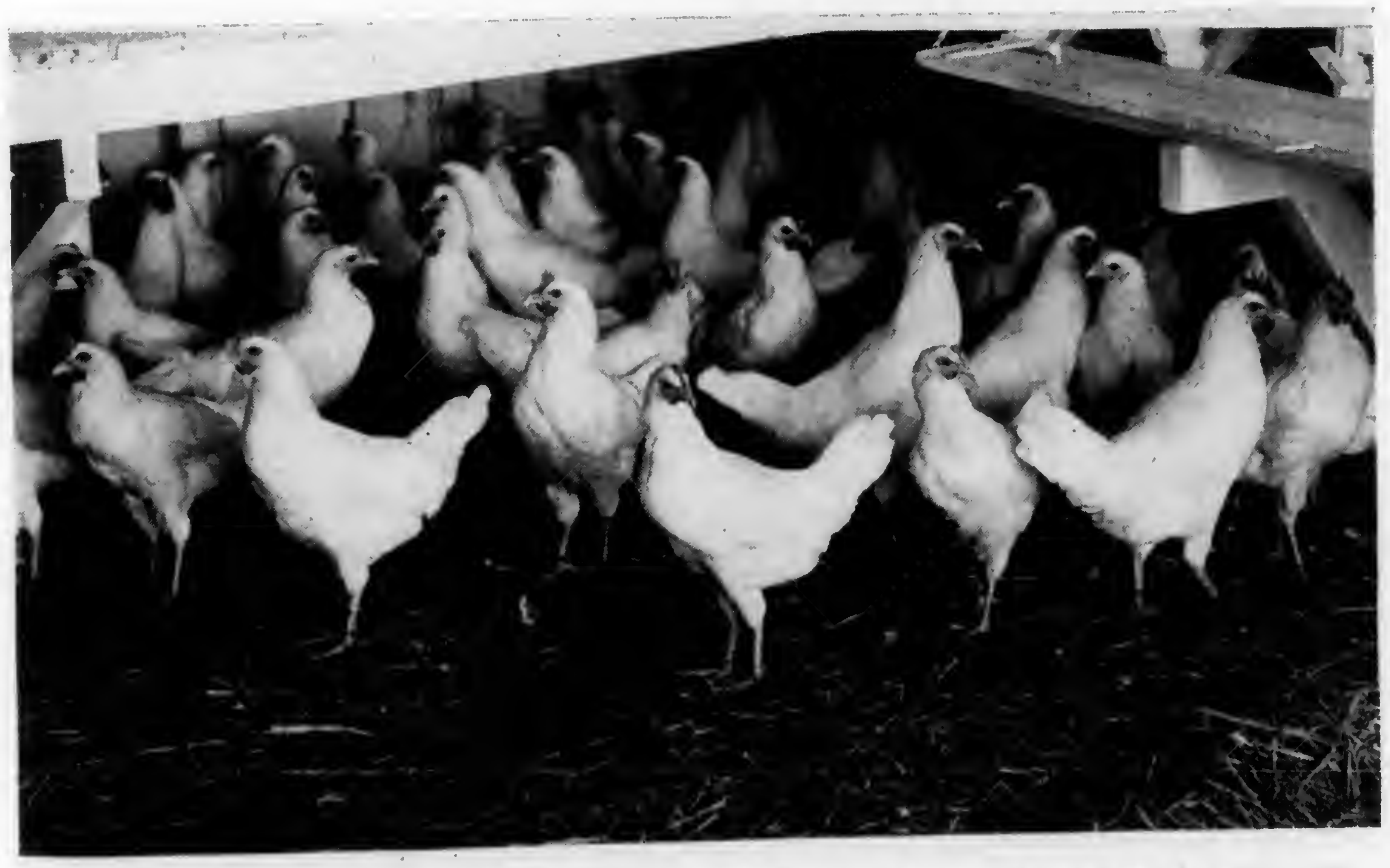


FIG. 2.—EXPERIMENTAL PULLETS, 16 WEEKS OLD, WHICH RECEIVED ADEQUATE AMOUNTS OF VITAMIN D

light was excluded from the pens in which the confined pullets were housed. All pens were of similar construction except the fronts of the two pens in which the range-reared pullets were housed. The fronts of these two pens were arranged to allow direct sunlight to enter. The pullets of the range-reared groups had access at all times to approximately one-fourth acre of alfalfa range.

Results During Period of Growth, 1932-33 Experiment

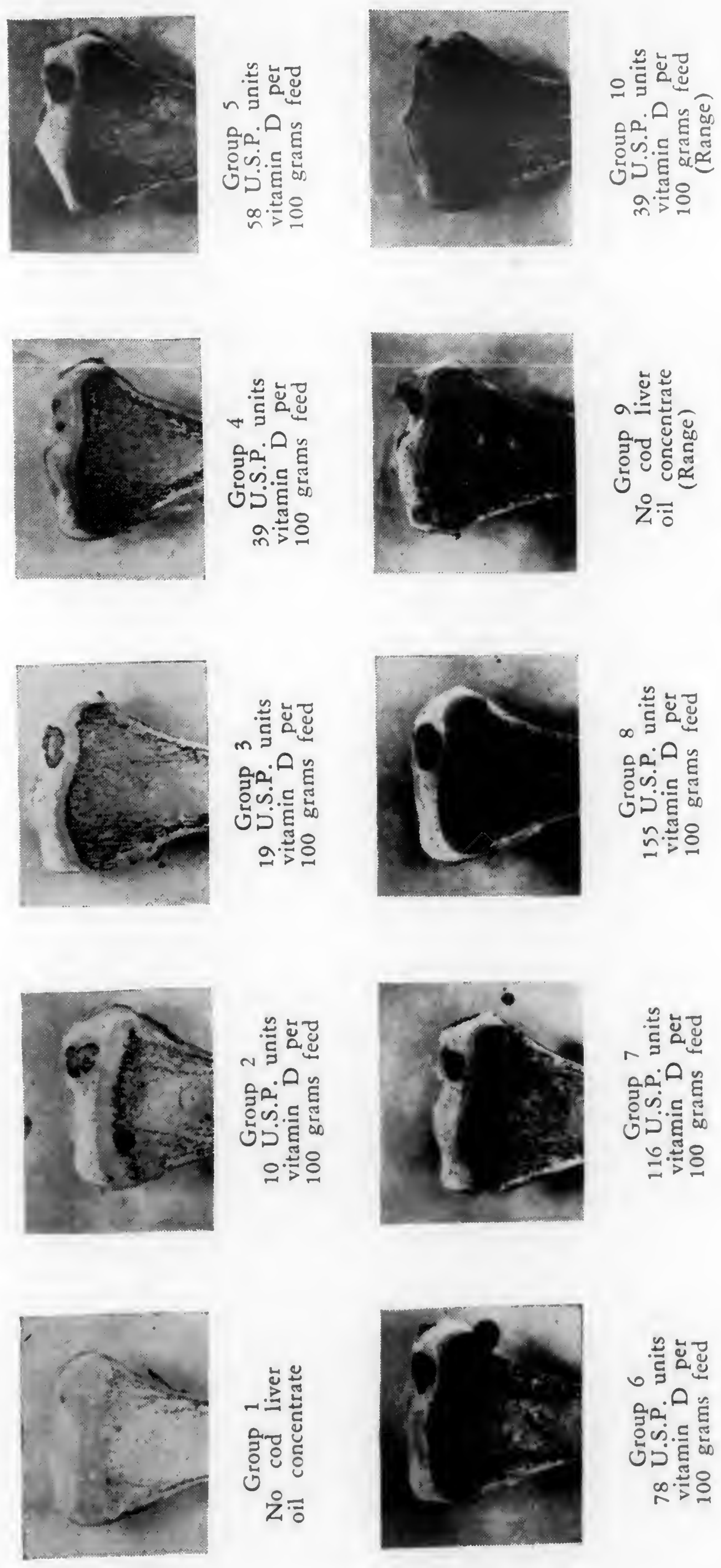
Body Weight—Chicks of all groups were individually weighed at time of hatching and at bi-weekly intervals for 16 weeks. From 16 weeks to the conclusion of the experiment individual weights were taken every four weeks. The weights were recorded in grams and the average weight per bird converted to pounds. During the growing period, the physical development, growth, feathering and pigmentation were satisfactory for all groups except Group 1 (no vitamin D) and Group 2 (10 U.S.P. units of vitamin D per 100 grams of feed). Figures 1 and 2 illustrate the marked differences existing at 16 weeks between pullets receiving inadequate amounts of vitamin D and those receiving adequate quantities of the vitamin.

Feed Consumption—With the exception of varying amounts of vitamin D, all groups received the same ration. All feed consumption was recorded at bi-weekly intervals for the first 16 weeks and at four week intervals thereafter. When the pullets were moved to laying quarters the feed consumption decreased markedly from the 12th to the 14th weeks. Several days were required for the birds to become accustomed to the new environment. With the exception of Group 2, all groups consumed approximately the same amount of feed. Group 1 was discontinued at the end of the 16th week because of the extreme rachitic condition of all individuals. All birds in this group exhibited a severe diarrheal condition.

Vitamin D Intake—The cod liver oil concentrate used throughout this experiment was found to contain 310 U.S.P. units of vitamin D per gram. The potency of the concentrate was determined by the rat assay using a U.S.P. reference cod liver oil. In Table 14 of the appendix is given the average number of U.S.P. units of vitamin D consumed per pullet during the entire experiment.

Bone Ash Determinations—One of the objectives of this experiment was to study the effect of varying amounts of vitamin D on bone development in the chick. For measuring the degree of calcification occurring in the bones of chicks, three common methods were used: bone ash determinations, blood calcium determinations and bone photographs of the interior structure. It is a well established fact that the amount of ash contained in the bones of a rachitic chick is considerably less than that in the bones of a normal chick. Furthermore, the amount of calcium present in the blood serum of a rachitic chick is less than that present in the blood of a normal chick. Bone photographs taken during the early period of growth gave a reliable indication as to the degree of calcification occurring in the bones of chicks. Results of the bone ash determinations, blood calcium determinations and bone photographs are given in Table 1 and Figures 3 and 4.

The percentage of bone ash at 8, 16, and 24 weeks is the average of four tibiae taken from the right leg of four chicks from each group and ashed



Group 1
No cod liver
oil concentrate

Group 2
10 U.S.P. units
vitamin D per
100 grams feed

Group 3
19 U.S.P. units
vitamin D per
100 grams feed

Group 4
39 U.S.P. units
vitamin D per
100 grams feed

Group 5
58 U.S.P. units
vitamin D per
100 grams feed

Group 6
78 U.S.P. units
vitamin D per
100 grams feed

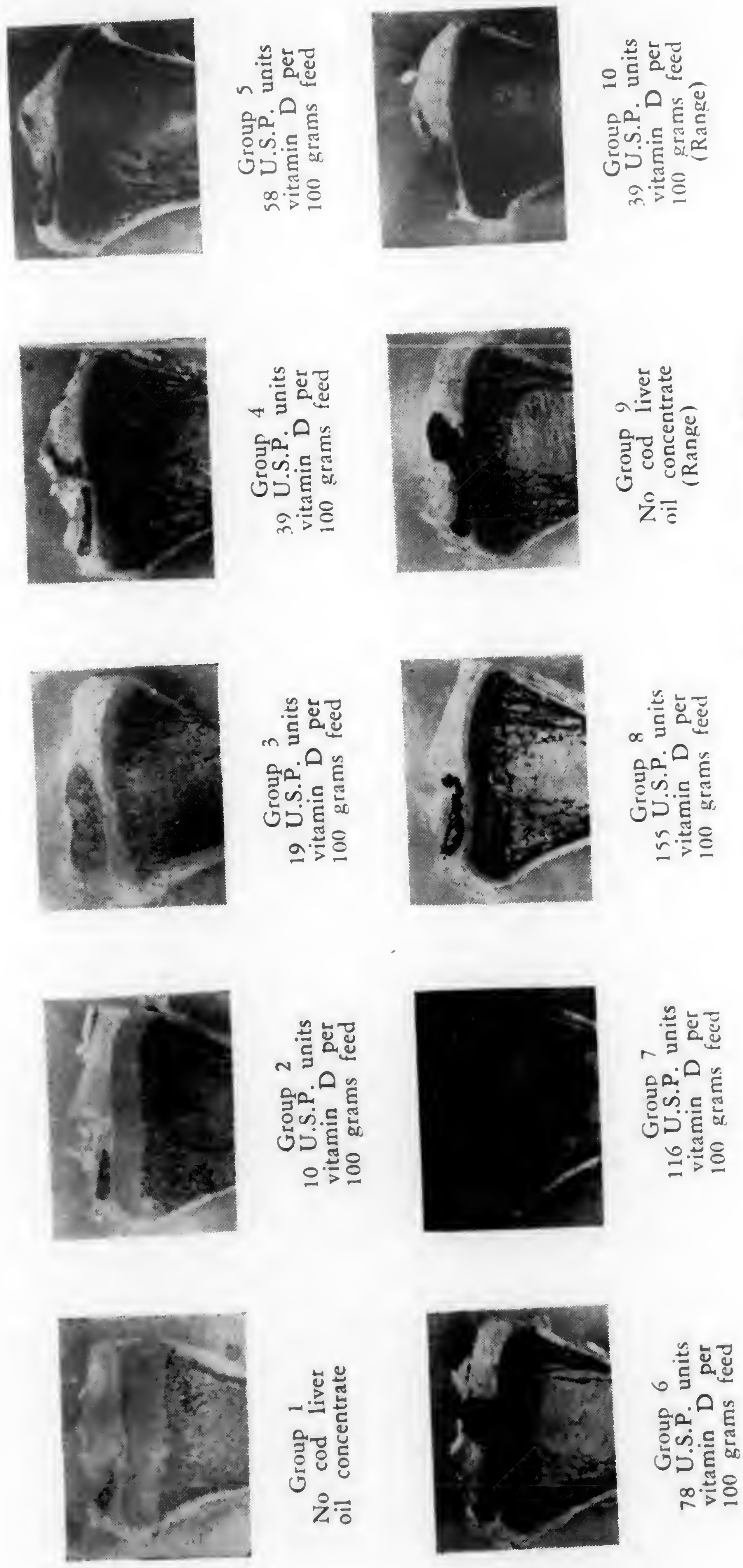
Group 7
116 U.S.P. units
vitamin D per
100 grams feed

Group 8
155 U.S.P. units
vitamin D per
100 grams feed

Group 9
No cod liver
oil concentrate
(Range)

Group 10
39 U.S.P. units
vitamin D per
100 grams feed
(Range)

FIG. 3.—INTERNAL STRUCTURE OF THE LEFT TIBIAE AT EIGHT WEEKS



Group 1
No cod liver
oil concentrate

Group 2
10 U.S.P. units
vitamin D per
100 grams feed

Group 3
19 U.S.P. units
vitamin D per
100 grams feed

Group 4
39 U.S.P. units
vitamin D per
100 grams feed

Group 5
58 U.S.P. units
vitamin D per
100 grams feed

Group 6
78 U.S.P. units
vitamin D per
100 grams feed

Group 7
116 U.S.P. units
vitamin D per
100 grams feed

Group 8
155 U.S.P. units
vitamin D per
100 grams feed

Group 9
No cod liver
oil concentrate
(Range)

Group 10
39 U.S.P. units
vitamin D per
100 grams feed
(Range)

Fig. 1.—Internal Structure of the Left Tibiae at Sixteen Weeks

separately. Four chicks weighing within a few grams of the average weight for the group were used. The bones were preserved in alcohol. All adhering muscle and connective tissues were removed but the proximal and distal cartilaginous caps were not removed. The bones then were dried in an oven at 105 degrees C for 24 hours. All bones were split longitudinally, placed in asbestos cones and extracted with hot 95 per cent alcohol for a period of 20 hours in a Soxhlet apparatus, followed by a similar treatment with ether. After extraction, the bones were dried to constant weight and burned in an electric muffle furnace to a white ash. Crucibles containing the bones were placed in a cold furnace and the temperature gradually increased until ashing was complete.

TABLE 1. SUMMARY OF RESULTS WITH GROWING CHICKS
1932–33 EXPERIMENT

Group number	1	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	0	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Weeks	Average body weight of pullets in pounds									
8	0.66	0.98	1.19	1.23	1.19	1.22	1.16	1.18	1.18	1.24
16	1.34	1.92	2.49	2.50	2.59	2.33	2.46	2.33	2.61	2.61
24	*	2.57	3.35	3.40	3.37	3.38	3.42	3.32	3.54	3.62
	Pounds of feed consumed per pullet to date									
0 to 8	2.58	3.68	3.63	3.70	3.55	3.61	3.49	3.56	3.76	3.97
0 to 16	6.26	7.95	10.10	10.35	10.38	10.18	9.90	10.03	10.95	11.30
0 to 24	*	15.01	19.46	19.67	19.65	19.64	10.28	20.03	19.97	21.48
	Intake of U.S.P. units of vitamin D from ration per pullet to date									
0 to 8	0	136	320	649	936	1,267	1,841	2,497	0	697
0 to 16	0	351	889	1,819	2,739	3,576	5,223	7,089	0	1,984
0 to 24	*	661	1,712	3,457	5,183	6 902	10,171	14,119	0	3,774
	Average per cent ash of tibiae									
8	39.2	44.6	51.1	51.1	51.2	52.1	52.2	51.7	52.5	52.5
16	47.1	50.9	51.0	52.9	52.5	55.0	53.6	52.6	54.7	54.9
24	*	49.2	56.5	56.4	54.5	57.0	55.9	54.8	56.8	54.7
	Milligrams of calcium per 100 cubic centimeters of blood serum									
8	4.8	7.5	9.4	11.4	10.3	11.5	10.5	10.3	10.9	10.4
16	5.6	8.6	9.5	10.3	10.6	11.5	12.2	11.4	11.1	12.3
24	*	10.4	11.6	11.8	11.9	11.8	12.0	11.9	11.6	11.9

* Group 1 was discontinued at 16 weeks.

Blood Serum Calcium Determinations—Serum calcium was determined on a composite sample of blood taken from the four chicks of each group used for bone ash determinations. By means of heart puncture, four cubic centimeters of blood were taken from each chick. The blood samples from the four chicks of each group were pooled for analysis. Some of the pullets were in egg production at 24 weeks of age but blood samples were taken only from those not in production. The Clark-Collip modification of the Kramer-Tisdall method was used for determining the serum calcium of the samples.

Bone Photograph Studies—At 8, 16, and 24 weeks of age, studies were made of the internal structure of the left tibiae of those chicks used in the bone ash determinations. The split proximal end of each left tibia was photographed to show the internal structure. At eight weeks of age, the four bones from Group 1 (no vitamin D) were diagnosed as being definitely rachitic. One bone in Group 2 (10 U.S.P. units of vitamin D per 100 grams of feed) was rachitic, two showed slight rachitic lesions, and one showed slight deviation from normal. In Group 3 (19 U.S.P. units of vitamin D per 100 grams of feed) three bones showed slight deviation from normal and one bone was normal. All bones in the remaining groups showed normal internal structure (Fig. 3).

At 16 weeks, the bone photographs showed all bones of Group 1 (no vitamin D) to be definitely rachitic. Two of the bones in Group 2 (10 U.S.P units of vitamin D per 100 grams of feed) were diagnosed as rachitic and two indicated a slight deviation from normal. All of the bones of Group 3 (19 U.S.P. units of vitamin D per 100 grams of feed) were normal except one and it showed slight rachitic lesions. In the remaining groups the bones showed normal internal structure (Fig. 4). At this time (16 weeks) Group 1 was discarded.

When the pullets were 24 weeks of age, all bones which were examined were diagnosed as normal with the exception of Group 2 (10 U.S.P. units of vitamin D per 100 grams of feed). One bone in this group was definitely rachitic, two showed slight deviations from normal, and the fourth bone was normal according to internal structure studies.

Mortality—For the first 24 weeks of this experiment the mortality was not excessive in any of the groups except Group 1 (no vitamin D). A large part of the mortality in this group during the 16 weeks the pullets were on experiment was caused by rickets.

Summary of Results with Growing Chicks, 1932-33 Experiment

It is evident from the manner in which Group 1 (no vitamin D) reacted that the basal rations used during the period of growth were definitely rachitogenic. The first external symptoms of rickets in this group were observed when the chicks were three and one-half weeks old. By the time they were ten weeks of age all chicks in this group showed marked external symptoms of rickets. The chicks of Group 2 (10 U.S.P. units of vitamin D per 100 grams of feed) developed noticeable external symptoms of rickets at about seven weeks of age. When 14 weeks old, all showed marked indications of a rachitic condition. Crooked breast bones were quite common. At 24 weeks, the breast bones of all pullets of the group were very crooked. Data on growth, bone ash and blood calcium (Table 1) indicate that Group 2 (10 U.S.P. units of vitamin D per 100 grams of feed) did not receive sufficient vitamin D for the normal development of a pullet.

Under the conditions of this experiment it is doubtful if Group 3 (19 U.S.P. units of vitamin D per 100 grams of feed) received sufficient vitamin D to protect the pullets against rickets. The internal structure of the bones at 8 and 16 weeks (Figs. 3 and 4) showed a slight deviation from normal. The low blood calcium and bone ash values at these same periods (Table 1) indicated a slight rachitic condition among the individuals of the group. Only slight differences were observed in the pullets of the five remaining confined groups fed larger amounts of vitamin D as evidenced by growth, bone ash, blood calcium and general appearance.

At no time during the growing period were external symptoms of rickets apparent in any of the groups except Groups 1 and 2. From hatching time to 24 weeks of age the results indicate that 39 U.S.P. units of vitamin D per 100 grams of feed when added to an all mash ration is the effective level under the most adverse conditions. When equal parts of grain and mash are fed, the latter should contain 78 U.S.P. units of vitamin D per 100 grams. In the first 24 weeks, each pullet when fed 39 U.S.P. units of vitamin D per 100 grams of feed consumed approximately 3,500 U.S.P. units of vitamin D.

The addition of 39 U.S.P. units of vitamin D per 100 grams of feed to the ration of Group 10 which had access to sunlight did not seem to have any appreciable effect on the degree of bone calcification or on the growth of the pullets during the rearing period of 24 weeks. The pullets of Group 9 which were entirely dependent upon direct sunlight as a source of vitamin D produced results which were as satisfactory as those of Group 10 which received additional vitamin D in the ration. It should be emphasized that both groups of pullets had access to sunlight, when available, throughout the growing period; a practice which is not ordinarily followed under farm conditions.

Experimental Procedure, 1934-35 Experiment

For the 1934-35 experiment, 1,575 Single Comb White Leghorn chicks of the Pennsylvania State College strain, hatched November 22, 1933 were used. Nine groups each containing 175 selected chicks were weighed, wing banded and placed in individual brooding pens. The chicks were confined in 12 by 20-foot pens in a long continuous type of brooder house. The pens were similarly constructed and equipped. In order to exclude direct sunlight the windows of each pen were covered with a soap compound.

Each group of chicks was fed the same basal all-mash rations as were used in the 1932-33 experiment. To the ration of each group were added the following amounts of cod liver oil concentrate.

Group number	*Per cent cod liver oil concentrate in total ration*	*U.S.P. units vitamin D per 100 grams feed*
1	0	0
2	1/16	19
3	1/8	39
4	3/16	58
5	1/4	78
6	5/16	97
7	3/8	116
8	1/2	155
9	1	310

All cockerels were removed at an early age. On account of the severe rachitic condition of the pullets in Group 1 (no vitamin D) this group was discontinued at the end of the eighth week. Between the 12th and 13th weeks of the experiment the starting mash was replaced with the growing and laying mash. At 16 weeks of age, 55 representative pullets from each of the remaining eight groups were selected for the laying pens. Body weight, health, condition, physical development and state of maturity were considered in the selection. The pullets were housed in the same laying house and kept under the same environmental conditions as were the confined pullets of the 1932-33 experiment. Precautions were taken to prohibit any ultra-violet light from entering the pens in which the pullets were confined.

Under the discussion of the 1932-33 results, methods were described by which data on growth, feed consumption, vitamin D intake, bone ash, and blood serum calcium were obtained. The same method of procedure in obtaining these same data was followed in the second experiment. Table 2 gives a summary of the results obtained during the growing period of the 1934-35 experiment. Detailed data are given in the appendix.

Summary of Results with Growing Chicks, 1934-35 Experiment

During the growing period the physical development, feathering and pigmentation were satisfactory in all groups except Group 1 (no vitamin D). External symptoms of rickets were first observed in Group 1 when the chicks were approximately four weeks old. Most of the chicks of the group at eight weeks of age were severely rachitic (Fig. 5).

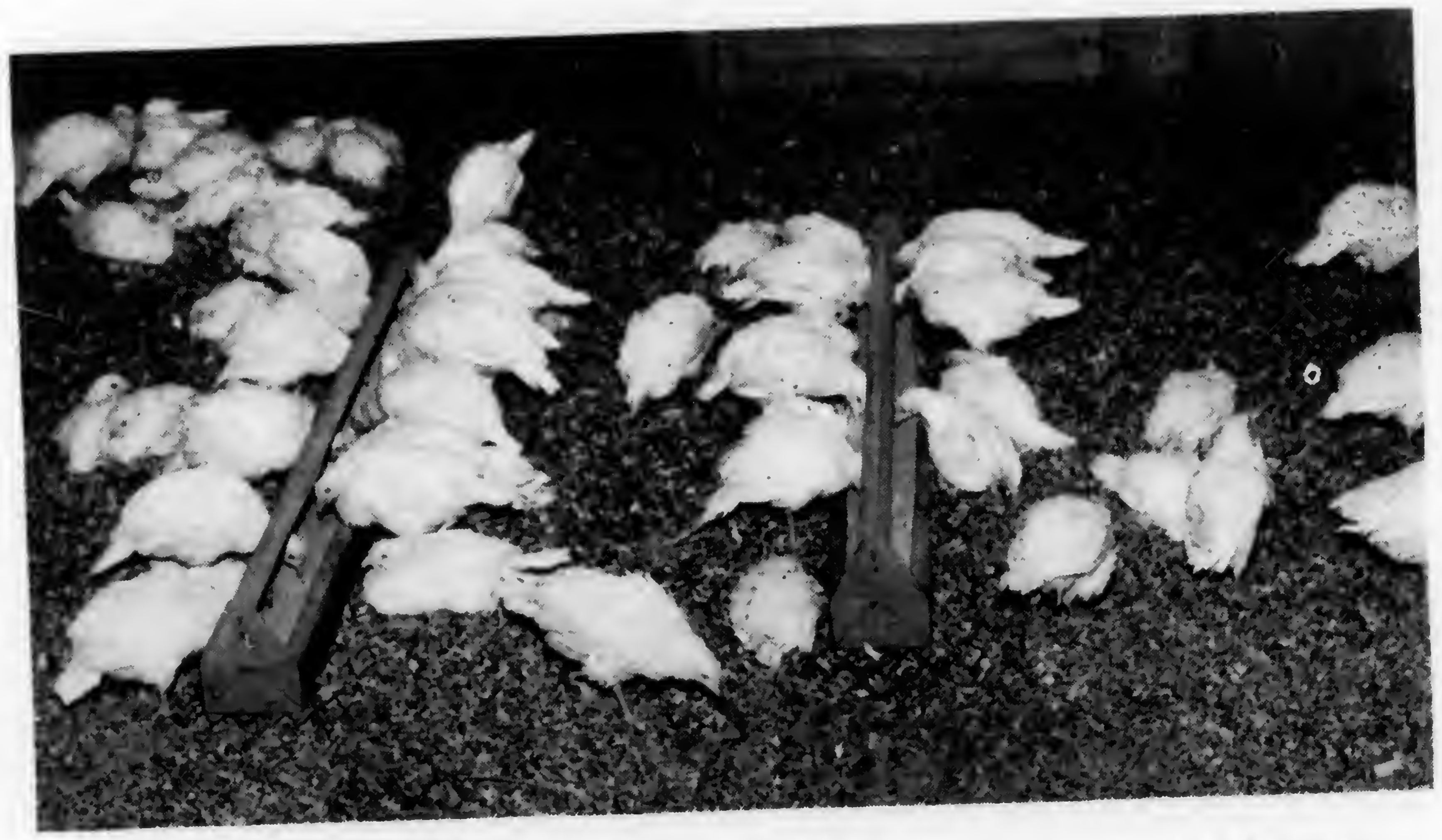


FIG. 5.—EXPERIMENTAL PULLETS, 8 WEEKS OLD, WHICH RECEIVED INADEQUATE AMOUNTS OF VITAMIN D

At eight weeks studies were made of the internal structure of the left tibiae of four chicks from each group. Two bones from Group 1 (no vitamin D) were diagnosed as being definitely rachitic, one showed slight rachitic lesions, and one bone was normal.

Growth was not satisfactory and bone ash and blood calcium values were below normal in Group 1 at eight weeks of age (Table 2). These results are in agreement with the observations made from the bone photographs. It is evident from the way in which Group 1 (no vitamin D) reacted that the basal rations used during the period of growth were definitely rachitogenic. There did not seem to be any particular objective to be gained by continuing this group. Consequently it was discarded from the experiment at the end of the eighth week.

TABLE 2. SUMMARY OF RESULTS WITH GROWING CHICKS
1934-35 EXPERIMENT

Group number	1	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	0	19	39	58	78	97	116	155	310
Weeks	Average body weight of pullets in pounds								
8	0.65	1.14	1.22	1.23	1.19	1.25	1.25	1.26	1.26
16	*	2.62	2.76	2.76	2.63	2.65	2.73	2.69	2.65
24	----	3.40	3.53	3.59	3.42	3.53	3.65	3.60	3.65
	Pounds of feed consumed per pullet to date								
0 to 8	2.74	3.90	3.91	4.02	3.84	3.92	3.88	4.20	3.95
0 to 16	*	11.94	12.67	12.88	12.27	12.49	12.58	13.00	12.74
0 to 24	----	24.20	25.58	26.04	24.73	25.86	25.74	26.42	25.75
	Intake of U.S.P. units of vitamin D from ration per pullet to date								
0 to 8	0	342	687	1,057	1,347	1,720	2,041	2,954	5,550
0 to 16	*	1,048	2,227	3,390	4,307	5,487	6,620	9,136	17,898
0 to 24	----	2,125	4,495	6,860	8,686	11,363	13,556	18,570	36,189
	Average per cent ash of tibiae †								
8	45.2	52.3	53.7	53.6	53.9	55.0	55.3	53.9	53.0
16	*	55.2	57.3	57.4	56.8	57.5	58.1	56.7	56.1
24	----	----	----	----	----	----	----	----	----
	Milligrams of calcium per 100 cubic centimeters of blood serum ‡								
8	8.2	10.2	11.2	11.5	11.9	11.9	11.6	11.8	11.3
16	*	10.0	11.3	12.1	12.1	12.0	11.8	10.9	11.4
24	----	24.6	25.1	25.1	27.2	26.7	26.9	30.0	27.0

* Group 1 was discontinued at 8 weeks.
† Per cent bone ash was not determined at 24 weeks.
‡ Calcium determinations at 24 weeks were made from pullets in production.

From the bone photographs taken at eight weeks one bone from Group 2 (19 U.S.P. units of vitamin D per 100 grams of feed) showed a slight deviation from normal and three bones were diagnosed as normal. All bones in the remaining groups were normal. Bone ash percentages at 8 and 16 weeks and blood calcium values at 8, 16 and 24 weeks (Table 2) are slightly lower for Group 2 than for the remaining groups supplied larger amounts of vitamin D. Many of the pullets of Group 2 at 24 weeks of age had developed deformed breast bones. These results are in agreement with those of the 1932-33 experiment. Nineteen U.S.P. units of vitamin D per 100 grams of feed did not appear to be sufficient to completely protect confined pullets against rickets.

As evidenced by growth, bone ash, blood calcium, internal structure of bone, general appearance (Fig. 6), and mortality only slight differences were observed in the pullets of the seven remaining groups supplied larger amounts of vitamin D from cod liver oil concentrate. Results obtained in the 1932-33 and the 1934-35 experiments indicate that 39 U.S.P. units of vitamin D per 100 grams of feed when added to an all-mash ration is the effective and economical level for chicks from hatching time to maturity under the most adverse conditions. When equal parts of grain and mash are fed, the mash should contain 78 U.S.P. units of vitamin D per 100 grams.

During the first 24 weeks of the 1934-35 experiment, each pullet when supplied 39 U.S.P. units of vitamin D per 100 grams of feed consumed approximately 4,500 U.S.P. units of vitamin D as compared to 3,500 U.S.P. units consumed by the pullets on the same level of oil during the same period of the 1932-33 experiment. This difference was due to the increase in feed consumption. The pullets of the 1934-35 experiment were reared during the fall and winter months while those of the 1932-33 experiment were reared during the spring and summer months. Also the pullets of the first experiment were brooded for 12 weeks in batteries while those of the last experiment were confined to the floor throughout the growing period.

FIG. 6.—EXPERIMENTAL PULLETS, 8 WEEKS OLD, WHICH RECEIVED ADEQUATE AMOUNTS OF VITAMIN D

Results During Laying Period, 1932-33 Experiment

When the pullets were 24 weeks of age, the number in each group was reduced to 50 representative individuals. At that time, only 49 pullets remained in Group 2 (10 U.S.P. units of vitamin D per 100 grams of feed). The mash used during the growing period (12 to 24 weeks) also served as the laying mash. No calcium supplements were fed to any of the groups. An insoluble grit was constantly available in hoppers.

Mash consumption was recorded in 4-week intervals. The number of pounds of mash consumed per pullet was calculated on a hen-day basis and recorded as the average feed consumption per pullet. Three cockerels were added to each group during the breeding season, which extended from the 40th through the 60th week. The feed consumption and the average number of U.S.P. units of vitamin D consumed per pullet during each 4-week period of the laying year are given in Tables 12 and 14 of the appendix. Beginning October 1, 1932, morning lights were used in the laying pens and the birds were provided a 12-hour day.

Body Weight of Pullets—The pullets of each group were individually weighed every four weeks. From the 28th to the end of the 36th week, no group showed any appreciable increase in body weight (Table 10). A rather marked decrease in egg production occurred during the period from 32 to 36 weeks. At 36 weeks, artificial illumination was increased to provide the pullets with a 13-hour day. In addition, a portion of the dry mash that the birds ordinarily received was moistened with water to a crumbly consistency and fed daily at noon. It is obvious from the weight of the birds at 40 weeks that these two changes in management had the desired effect. Maximum body weight of the pullets was reached in all groups at 40 weeks of age.

Egg Production—All pullets were trapnested throughout the laying year. Calculations determining the per cent egg production and number of eggs laid per pullet were made on a hen-day basis. A rather marked decrease in egg production occurred between the 32d and 40th weeks (Table 16). After the length of day had been increased and moist mash fed, egg production increased rapidly. From Table 3, it appears that egg production increased as the amount of vitamin D from cod liver oil concentrate was increased in an otherwise adequate laying ration. Results indicate that 78 U.S.P. units of vitamin D per 100 grams of feed as fed to Group 6 was optimum for egg production. When the optimum level was reached further additions of vitamin D did not significantly increase egg production.

TABLE 3. SUMMARY OF RESULTS WITH LAYING PULLETS *
1932-33 EXPERIMENT

Group number	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Body weight in pounds per pullets	3.02	3.56	3.54	3.64	3.69	3.72	3.56	3.75	3.78
Per cent egg production -------	7.6%	16.6%	39.9%	38.6%	47.4%	48.8%	44.7%	44.0%	45.4%
Number of eggs per pullet --------	28	61	145	141	173	178	163	160	165
Weight of eggs in grams † -------	51.0	50.9	51.7	52.7	52.3	52.7	52.3	53.1	52.9
Per cent shell of egg ------------	6.7%	7.0%	8.7%	8.9%	8.8%	9.0%	9.2%	9.1%	9.0%
Per cent ash in egg shell ------	52.1%	52.4%	53.0%	53.5%	53.2%	53.2%	53.2%	53.2%	53.1%
Milligrams calcium per 100 cc serum -	18.7	21.4	24.3	24.7	25.5	26.0	25.2	26.6	25.5
Per cent hatch of fertile eggs ------	56.4%	44.5%	74.6%	84.5%	84.9%	88.1%	87.8%	91.0%	83.3%

* The data given were taken from Tables 10, 16, 18, 20, 22, 24 and 26 of the appendix and are averages for 52 weeks of egg production.

† A 2-ounce egg weighs 56.7 grams.

Weight of Eggs and Shell Quality—The total daily egg production was weighed by groups. The average egg weight for each 4-week period is given in Table 18 of the appendix. It appears that adequate amounts of vitamin D tend to increase egg size. Furthermore, these studies indicate that when sufficient amounts of vitamin D are supplied to laying hens, heavier egg shells are produced (Table 3). Complete data obtained from ten eggs of each group at 12 times during the laying year are given in Table 20 of the appendix. The eggs were weighed individually on analytical balances the day they were laid. After weighing, they were broken and the albumin washed out of the shells with distilled water. The shells from the ten eggs of each group were pooled, pulverized in a mortar, dried to a constant weight, and weighed. A 2-gram sample from each composite sample was ashed. The percentages of ash found in the egg shells are given in Table 22. Although differences in the per cent of ash contained in the egg shells from the various groups were not large, it appears that less mineral matter was deposited in the shells of eggs laid by pullets on the lower levels of vitamin D (Table 3). In grinding the egg shells from the various groups for ashing purposes, it was observed that a marked difference in the degree of hardness existed between the shells of eggs laid by pullets on low intakes of vitamin D and those from eggs laid by pullets fed larger amounts of vitamin D. The shells from eggs laid by pullets receiving ample vitamin D were considerably harder and consequently more difficult to grind than were those from eggs laid by pullets on the lower levels of vitamin D.

Blood Serum Calcium—The results of 12 calcium determinations of the blood serum made during the year in each of the groups are given in Table 24. These determinations were made on a composite sample of blood from five birds of each group. All birds from which samples of blood were taken were in production. Four cubic centimeters of blood were removed from each pullet by means of a hypodermic syringe inserted into the brachial vein of the left wing. The serum calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method. The serum calcium content of the blood varied considerably throughout the laying period as shown in Table 24. The average of the 12 serum calcium determinations showed that only slight differences existed among the various groups, except for Groups 2 and 3 on low levels of vitamin D. In these two groups the calcium content of the blood serum was low.

Mortality—During the latter part of this experiment, the mortality was rather excessive particularly in Group 2 (10 U.S.P. units of vitamin D per 100 grams of feed). There was no significant difference in mortality among the other groups.

Hatchability—Hatchability data were secured from the pens fed various amounts of vitamin D. Five settings of eggs from each of the nine groups of pullets were incubated. The hatching period extended from March 10, 1933 to June 16, 1933. Three cockerels were placed with each group of pullets three weeks prior to the time hatching eggs were saved. The cockerels were rotated from pen to pen twice weekly during the breeding season. The eggs for incubation purposes were individually pedigreed. Hatching eggs were saved for one week previous to setting, and so far as possible, three eggs and never more than five were set from each pullet. For each hatch, 100 eggs from each group of pullets were incubated, with the exception of Groups 2 and 3 each of which produced less than this number of eggs during the period. Eggs laid nearest to the time of setting were selected for incubation purposes. The results of the five hatches are given in Table 26. Fertility was satisfactory, averaging 80 per cent or higher, in all groups except Group 8. For some unknown reason, fertility in this group was consistently low in each of the five hatches (67 per cent). As evidenced by the hatching results, there was a relationship between the amount of vitamin D supplied to breeders in the form of cod liver oil concentrate and the per cent hatch of fertile eggs (Table 3).

Transmission of Vitamin D to the Offspring—To determine the effect on the chick when varying levels of vitamin D were fed to the mother hen, all normal chicks from hatches 2, 3, and 5 (Table 26) were brooded for a period of eight weeks. Chicks from hatches 1 and 4 were not brooded, but were used only for hatchability studies. The chicks from hatches 2, 3 and 5 were weighed by groups at hatching time and bi-weekly thereafter for a period of eight weeks.

A basal ration of the same composition as that fed to the parent stock during their brooding period was fed to the progeny. All groups of hatch 2 received, in addition to the basal ration, 19 U.S.P. units of vitamin D per 100 grams of feed. This amount had been found in the work of the previous year to be the minimum protective level. The source of vitamin D supplied to the progeny was from the same lot of cod liver oil concentrate fed to the parent stock. No cod liver oil concentrate was fed to any of the groups of hatch 3. The chicks of hatch 5 were fed the same number of U. S. P. units of vitamin D per 100 grams of total ration as were the parent stock from which they were hatched (see page 4). All groups of chicks from hatches 2 and 3 and Groups 3 to 8 inclusive of hatch 5 were brooded in battery brooders and deprived of direct sunlight. In hatch 5, the chicks from Groups 9 and 10 were brooded in colony houses with access to range.

Data were obtained on growth, feed consumption, mortality, blood calcium, and bone ash of 1,551 progeny. There was no apparent evidence that the vigor of the chicks was influenced by the vitamin D intake of the parent stock. The results indicated that in order to determine the degree to which vitamin D is transmitted from the parent to the offspring more sensitive methods of measurement are needed.

Summary of Results with Laying Pullets, 1932-33 Experiment

Egg production was not satisfactory in Groups 2, 3, 4, and 5 which were fed 10, 19, 39, and 58 U.S.P. units of vitamin D per 100 grams of feed respectively. When all factors of measurement were considered, Groups 2 and 3 were materially inferior to any of the other groups. In maintenance of body weight, egg production, egg weight, per cent egg shell, and calcium content of blood, no apparent significant differences existed between Groups 6 and 7 which were fed 78 and 116 U.S.P. units of vitamin D per 100 grams of feed respectively. Egg production and body weight were not so satisfactory in Group 8 (155 U.S.P. units of vitamin D per 100 grams of feed) as they were in Groups 6 and 7. Groups 9 and 10, in which the pullets had access to range at all times exhibited only small differences but the egg production was less satisfactory than in confined Groups 6 and 7.

The evidence indicates that when laying pullets are denied access to direct sunlight, 78 U.S.P. units of vitamin D per 100 grams of total ration is the most effective level. In this experiment, the above number of U.S.P. units of vitamin D were supplied by feeding one-fourth per cent of a cod liver oil concentrate containing 310 U.S.P. units of vitamin D per gram. This amount will supply sufficient vitamin D to fulfill adequately the requirements of laying pullets maintained under the most adverse conditions. When laying pullets, subjected to environmental conditions similar to those employed in this study, are fed equal parts of mash and grain, 100 grams of the mash should contain 155 U.S.P. units of vitamin D.

Results During Laying Period, 1934-35 Experiment

On March 14, 1934 fifty-five representative 16-week-old pullets from each of the eight groups were selected and placed in the laying pens. These pullets were housed in the same house and placed under a similar system of management as were the pullets of the 1932-33 experiment. Since the laying pens were considerably darker than the brooding pens, it was necessary to artificially illuminate the laying quarters for a period of six weeks to avoid a decrease in the rate of growth. The pullets were provided with a 13-hour day which was later increased to 14 hours. At the end of the 24th week the number of pullets in each group was reduced to 50. The mash fed during the growing period (12 to 24 weeks) also served as the laying mash.

The pullets of all groups when approximately 26 weeks of age (May 26, 1934) became infested with a virulent strain of coccidia. The disease first appeared in Groups 5, 6, and 7 and immediately spread to the other groups. At the time the disease appeared all groups except Group 2 (19 U.S.P. units of vitamin D per 100 grams of feed) and Group 3 (39 U.S.P. units of vitamin D per 100 gram of feed) were in excellent condition and in satisfactory egg production. The egg production of Group 4 (58 U.S.P. units of vitamin D per 100 grams of feed) was approximately 40 per cent. In Group 9 (310 U.S.P. units of vitamin D per 100 grams of feed) egg production was slightly less than 60 per cent. While in Groups 5, 6, 7, and 8 (78, 97, 116 and 155 U.S.P. units of vitamin D per 100 grams of feed respectively) the average egg production was approximately 50 per cent (Table 17).

Previous to this outbreak there had been no evidence at any time of coccidiosis in any of the groups. In order to avoid heavy losses all pullets in the entire experiment were fed a limited amount of moist mash and condensed buttermilk. The mash used as moist mash consisted of five per cent meat scrap added to equal parts of yellow corn meal, ground heavy oats, and wheat middlings plus the amount of cod liver oil concentrate supplied to the various groups. This treatment was of considerable benefit in checking the amount of blood appearing in the droppings.

On June 7, 1934, because of the appearance of some bloody droppings a 40 per cent milk flush treatment was given to all birds for a period of 24 hours. After this treatment, no blood was observed in the droppings. Immediately following the milk flush treatment, the pullets were given the experimental laying mash. This mash was fed for the remainder of the laying year.

For approximately four months after the outbreak of coccidiosis occurred, body weight, feed consumption, and egg production decreased. During the same period, the pullets of all groups molted. The groups on the high levels of the cod liver oil concentrate did not go through as severe or as complete a molt as did those on the lower levels. Groups 7, 8, and 9 (116, 155 and 310 U.S.P. units of vitamin D per 100 grams of feed respectively) did not fall below 40 per cent production during the period of molt (Table 17).

Throughout the fall and winter months, it was necessary to feed a moist mash to each group of pullets twice a day. The moist mash was made by using a measured amount of experimental dry mash with diluted condensed buttermilk. The condensed buttermilk was fed at the rate of eight ounces per pen per day. Twelve ounces of finely cut fresh green feed was added to the moist mash of each group and fed daily. After the 48th week, when fresh green feed was no longer available, cabbage at the rate of one pound per pen per day was fed. The amounts of all supplementary feeds given to the pullets such as condensed buttermilk, green feed and cabbage were converted to a dry weight and added to the amount of laying mash consumed during each 4-week period. Table 13 gives the total feed consumption for each 4-week period during the laying year.

Immediately following the infestation of coccidia mortality was rather excessive, particularly in the groups on the lower levels of vitamin D. The total per cent mortality for the entire year of 52 weeks ranged from 20 per cent in Group 9 (310 U.S.P. units of vitamin D per 100 grams of feed) to 60 per cent in Group 3 (39 U.S.P. units of vitamin D per 100 grams of feed). As the levels of cod liver oil concentrate increased even above the optimum level as determined in the 1932-33 experiment, the rate of mortality from coccidial infestation appeared to decrease.

On December 19, 1934 three male birds were placed in each of the eight pens. Similar to the practice followed in the 1932-33 experiment the male birds were rotated from pen to pen twice each week preceding and during the hatching season. The hatching period extended from March 6, 1935 to May 9, 1935. During this period, three hatches were made from each of the eight groups of pullets. Hatching eggs were individually pedigreed and were saved for a period of ten days previous to setting. In the groups of pullets which laid more than 100 eggs during the 10-day period the number of eggs set was limited to 100. As far as possible, equal numbers of eggs from each pullet were selected for each hatch. In the groups producing less than 100 eggs, the total production of the pen was used for incubation purposes. Table 27 gives the results obtained from the three hatches. Fertility was satisfactory but not as high as it was in the 1932-33 experiment. The fertility for the three hatches averaged 70 per cent or higher in all groups while in the previous experiment fertility averaged 80 per cent or better in all groups except one.

Similar methods to those of the 1932-33 experiment for collecting data on body weight, feed consumption, vitamin D intake, egg production, size of egg, quality of egg shell and blood serum calcium were followed in the 1934-35 experiment. These results are given in Tables 11, 13, 15, 17, 19, 21, 23, and 25 of the appendix.

The 1934-35 experiment extended over a period of 72 weeks which was four weeks less than the duration of the 1932-33 experiment. At 72 weeks of age the pullets had completed one year (52 weeks) of egg production. The pullets of the 1934-35 experiment were hatched in the fall and came into production approximately four weeks earlier than did the spring-hatched pullets of the 1932-33 experiment.

Transmission of Vitamin D to the Offspring—Studies on eggs produced by Groups 2 to 10 inclusive of the 1932-33 experiment and Groups 2 to 9 inclusive of the 1934-35 experiment showed that a marked relationship exists between the vitamin D potency of the egg yolk and the vitamin D intake of the hen producing it.[4] Because of the existence of this relationship, is seemed reasonable to expect a transfer of the vitamin from the hen to the chick which could be measured by the degree of bone calcification.

Since slight but no conclusive evidence had been secured in the 1932-33 experiment to indicate a transmission of vitamin D, the work was repeated in the 1934-35 experiment using a different experimental procedure.

The three hatches of chicks from each of the eight groups of pullets were brooded for a period of five weeks. All chicks were marked for identification and were brooded together in battery brooders without access to sunlight. The chicks were fed the experimental chick starter which was proven to be deficient in vitamin D in the 1932-33 and the 1934-35 experiments. Every seven days during the brooding period representative individuals were killed and the per cent bone ash determined on the right tibia. Bone photographs were made from each left tibia.

From the bone photographs and bone ash determinations of a total of 346 sets of bones evidence was secured to indicate that vitamin D was transmitted in a quantitative way from the hen to the chick. The vitamin D reserve of the chick was dependent upon the vitamin D intake of the parent stock and this evidence was secured when the chicks were from one to four weeks old. This explains why somewhat negative results were secured in the 1932-33 experiment.[5]

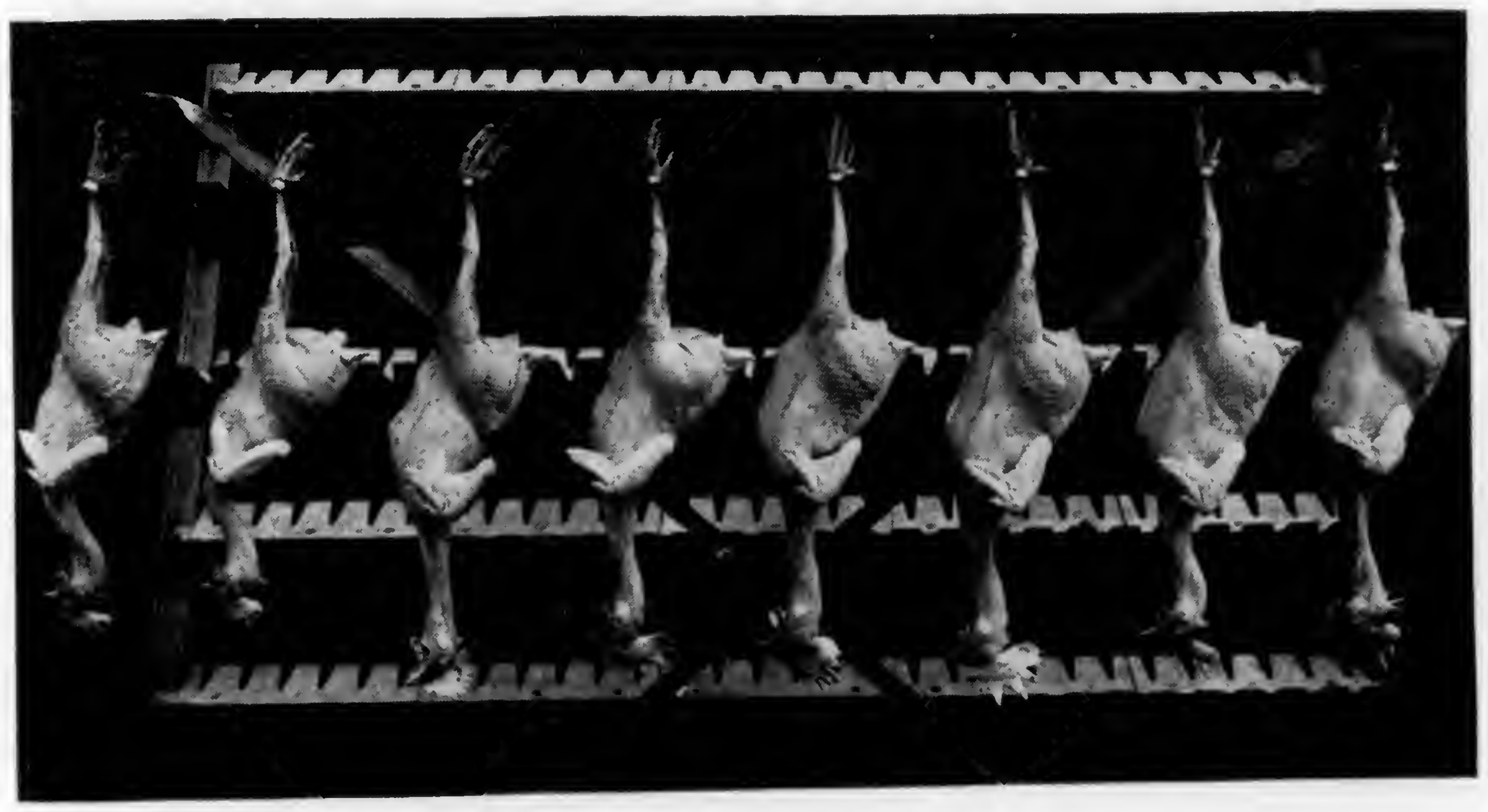
Effect of Vitamin D on Breast Bone Conformation—At the close of the 1934-35 experiment the pullets in all groups were examined and the condition of the breast bones noted. The breast bone of each pullet was classified according to four arbitrary degrees of deformity, namely: very deformed, deformed, slightly deformed and straight. Table 4 gives for each of the groups of pullets the percentages of the various degrees of deformity found in the breast bones. The condition of the breast bone of a representative pullet from each group is shown in Figure 7. There was a very definite relationship between the amount of vitamin D intake and the degree to which the breast bones of pullets were deformed.

Summary of Results with Laying Pullets, 1934-35 Experiment

Considering body weight, weight of eggs, quality of egg shell, blood serum calcium, and hatchability the results from the 1934-35 experiment confirm those of the 1932-33 experiment, Tables 3 and 5. The outbreak of coccidiosis which occurred shortly after the pullets had reached a satisfactory level of egg production very seriously affected the subsequent egg production in all groups except 8 and 9 fed 155 and 310 U.S.P. units of vitamin D per 100 grams of feed respectively. This amount of vitamin D is much higher than the optimum level for laying hens as shown in the 1932-33 experiment. It is possible that the coccidial infestation interfered with the efficiency of the utilization of the nutrients in the ration.

TABLE 4. CONFORMATION OF BREAST BONE OF PULLETS AT 72 WEEKS OF AGE 1934-35 EXPERIMENT

Group number	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	19	39	58	78	97	116	155	310
	Per cent deformity							
Very deformed	70.8	55.0	35.7	16.7	10.0	5.7	0.0	0.0
Deformed	20.8	20.0	50.0	33.3	33.3	20.0	20.6	7.5
Slightly deformed	8.3	20.0	7.1	33.3	30.0	42.9	41.2	30.0
Straight	0.0	5.0	7.1	16.7	26.7	31.4	38.2	62.5
	Per cent ash in tibia							
Average of 3 pullets	61.0	59 4	62.0	60.7	62.9	62.0	62.0	62.9



GROUP NUMBER

2 3 4 5 6 7 8 9

U. S. P. UNITS VITAMIN D PER 100 GRAMS FEED

19 39 58 78 97 116 155 310

FIG. 7. CONFORMATION OF BREAST BONE OF PULLETS AT 72 WEEKS OF AGE 1934-35 EXPERIMENT

The performance of the pullets in Groups 2 and 3 (19 and 39 U.S.P. units of vitamin D per 100 grams of feed) was markedly inferior to that of the pullets on higher levels of vitamin D. Egg size, per cent egg shell, and blood serum calcium (Table 5) were not so satisfactory in Group 4 (58 U.S.P. units of vitamin D per 100 grams of feed) as in the five remaining groups fed higher levels of cod liver oil concentrate. Except for egg production no apparent significant differences existed among Groups 5, 6, 7, 8, and 9 (78, 97, 116, 155, and 310 U.S.P. units of vitamin D per 100 grams of feed).

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[4] Pennsylvania Agricultural Experiment Station technical paper Number 686, "The Relationship of the Vitamin D Intake of the Hen to the Antirachitic Potency of the Eggs Produced." The Journal of Nutrition Vol 10, No. 2, pages 167 to 178.

[5] For detailed results obtained in both experiments on the transmission of vitamin D from the parent stock to the offspring, see Pennsylvania Agricultural Experiment Station Paper Number 700 "The Effect of Vitamin D Intake of the Hen on the Bone Calcification of the Chick." Poultry Science, Vol. 15 No. 4 Pages 284 to 289.

Results from both the 1932-33 and 1934-35 experiments indicate that the requirement of laying pullets denied access to sunlight is double that of growing chicks or 78 U. S. P. units of vitamin D per 100 grams of total ration. There did not seem to be any particular benefits to be derived from supplying more than this amount of vitamin D to laying pullets and breeding hens. However, egg production and mortality data of the 1934-35 experiment seem to indicate that the feeding of higher levels of cod liver oil concentrate to pullets is of considerable value in overcoming some of the detrimental effects brought about by a severe infestation of coccidia. More detailed results on this phase of the work will be published elsewhere.

TABLE 5. SUMMARY OF RESULTS WITH LAYING PULLETS*
1934-35 EXPERIMENT

Group number	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	19	39	58	78	97	116	155	310
Body weight in pounds per pullet	3.42	3.52	3.64	3.58	3.61	3.83	3.77	3.92
Per cent egg production	10.2%	14.5%	24.7%	35.3%	37.0%	43.4%	48.6%	48.8%
Number of eggs per pullet	37	53	90	129	135	158	177	178
Weight of eggs in grams †	43.1	46.2	48.4	49.8	50.7	50.5	51.2	51.0
Per cent shell of egg	6.1%	7.3%	8.1%	8.5%	8.9%	8.8%	9.1%	9.2%
Per cent ash in egg shell	51.7%	52.6%	53.0%	53.2%	53.4%	53.5%	53.4%	53.3%
Milligrams calcium per 100 cc serum	21.2	21.9	23.3	24.5	26.2	25.6	27.3	28.2
Per cent hatch of fertile eggs	11.1%	37.8%	77.4%	77.7%	79.6%	74.2%	86.5%	77.1%

* The data given were taken from Tables 11, 17, 19, 21, 23, 25 and 27 of the appendix and are averages for 52 weeks of egg production.

† A 2-ounce egg weighs 56.7 grams.

Seventy-eight U. S. P. units of vitamin D per 100 grams of total ration will adequately meet the requirements of confined laying pullets for vitamin D under normal conditions. When laying pullets are fed equal parts of mash and scratch grain, 100 grams of the mash should contain 155 U. S. P. units of vitamin D.

Range Groups, 1934-35 Experiment

On some farms growing chicks and laying hens have access to range and sunlight throughout the entire year. Information concerning the need of chicks and more particularly laying hens for vitamin D in addition to that supplied by exposure to direct sunlight is limited. For additional information two of the groups of pullets in the 1932-33 experiment had access to direct sunlight throughout the growing and laying periods. One group of pullets was fed a vitamin D deficient ration. The second group received 39 U. S. P. units of vitamin D per 100 grams of feed. As stated previously no apparent significant differences could be detected between the two groups of pullets. In the 1934-35 studies it seemed desirable to repeat the work following the same general procedure as in the previous experiment.

Experimental Procedure During Growing Period

Four groups, each containing 175 selected Single Comb White Leghorn chicks were used in this experiment. The chicks were hatched on April 25, 1934 and were immediately wing banded and weighed. Four colony brooder houses of similar construction were used for brooding purposes. The chicks were fed an all-mash ration of the same composition as fed in the previous experiments. To the mash was added the following percentages of the cod liver oil concentrate containing 310 U. S. P. units of vitamin D per gram.

Group number	*Per cent cod liver oil concentrate in total ration*	*U.S.P. units vitamin D per 100 grams feed*
10	None	0
11	1/16	19
12	1/8	39
13	1/4	78

When the chicks were 10 days old each group was given access to a limited range which was kept bare by frequent harrowing. Each range was heavily limed before harrowing. As a precautionary measure against coccidiosis a 40 per cent milk mash was fed to all groups of chicks for a period of 24 hours when they were three and one-half weeks old. This treatment was repeated every two weeks for a period of 16 weeks. No external symptoms of coccidiosis were observed among the pullets during the growing and subsequent laying periods. The 40 per cent milk flushing mash contained the same amount of cod liver oil concentrate as the starting and growing mashes for each group. The amounts of flushing mash consumed were included in the total feed consumption records.

The cockerels were removed from each of the groups at four weeks of age. The starting mash was replaced with the growing mash at the end of the 12th week. The growing mash which also served as the laying mash was fed from the 12th week to the conclusion of the experiment.

Fifty-five representative pullets from each of the groups were selected for the laying pens when 16 weeks of age. The pullets were selected on the same basis as had been followed in previous selections. They were housed in pens adjoining those containing the fall-hatched confined groups of the 1934-35 experiment. In the front and rear of each of the four pens were ranges of approximately one-fourth acre. The ranges were sown to oats and used alternately. The management of the four groups of pullets was the same as that of the pullets having access to range and sunlight in the 1932-33 experiment.

Summary of Results with Growing Chicks

Table 6 gives a summary of the results obtained during a growing period of 24 weeks from pullets maintained under range conditions. In obtaining these data the same method of procedure as used in the work with the confined groups was followed. At no time during the 24-week growing period were external symptoms of rickets observed in any of the groups. The physical development, rate and condition of feathering and pigmentation of all pullets on experiment were very satisfactory.

TABLE 6 SUMMARY OF RESULTS WITH GROWING CHICKS
RANGE GROUPS, 1934-35 EXPERIMENT

Group number	10	11	12	13
U.S.P. units vitamin D per 100 grams feed	0	19	39	78
Weeks	Average body weight of pullets in pounds			
8	1.20	1.22	1.24	1.20
16	2.78	2.83	2.82	2.76
24	3.52	3.59	3.59	3.53
	Pounds of feed consumed per pullet to date			
0 to 8	4.12	4.24	4.11	3.94
0 to 16	12.03	13.19	12.15	12.18
0 to 24	21.42	23.28	21.61	21.77
	Intake of U.S.P units of vitamin D from ration per pullet to date			
0 to 8	0	373	721	1,382
0 to 16	0	1,160	2,132	4,277
0 to 24	0	2,047	3,794	7,649
	Average per cent ash of tibiae *			
8	52.7	52.4	52.5	51.7
16	57.5	58.6	57.1	56.8
24	----	----	----	----
	Milligrams of calcium per 100 cubic centimeter of blood serum †			
8	11.6	11.8	11.5	11.6
16	12.3	11.9	11.9	12.1
24	29.1	26.1	25.6	27.4

* Per cent bone ash was not determined at 24 weeks.
† Calcium determinations at 24 weeks were made from pullets in production.

No marked differences appeared at any time during the growth period between the body weight of pullets in Groups 11, 12, and 13 fed 19, 39, and 78 U. S. P. units of vitamin D per 100 grams of feed respectively and the body weight of pullets in Group 10 which were entirely dependent upon direct sunlight as a source of vitamin D. At 8, 16, and 24 weeks the per cent ash in the tibia and the milligrams of calcium in the blood serum of pullets in Group 10 compared favorably with those of the other groups receiving the additional vitamin D from the cod liver oil concentrate (Table 6).

When pullets are reared under conditions similar to those employed in this study it appears that they can secure enough vitamin D by irradiation from direct sunlight for normal growth and bone calcification. However, it should be emphasized that the pullets of this experiment had access to direct sunlight, whenever available, from the time they were ten days of age to the completion of the growing period (24 weeks). Furthermore, these chicks were hatched in April and developed during the period of the year in which the greatest amount of ultra-violet light is obtained from the sun.

Experimental Procedure During Laying Period

At 24 weeks of age the number of pullets in each group was reduced to 50. Artificial morning illumination was started on September 13, 1934. The length of day was the same as given to the confined pullets. With the exception of the feeding of a moist mash the management of the groups on range was the same as the groups in confinement. A moist mash was not fed to the range groups during the laying year except for a short period toward the end of the experiment. When the supply of fresh green feed became inadequate on range, cabbage was fed at the rate of one pound per pen per day. This practice was continued until the pullets were approximately 46 weeks old. The laying mash which was fed throughout the laying period was of the same composition as the growing mash fed from the 12th to the 24th week. Three male birds were placed in each of the pens on December 19, 1934 and were rotated as were those in the confined pens.

Summary of Results with Laying Pullets

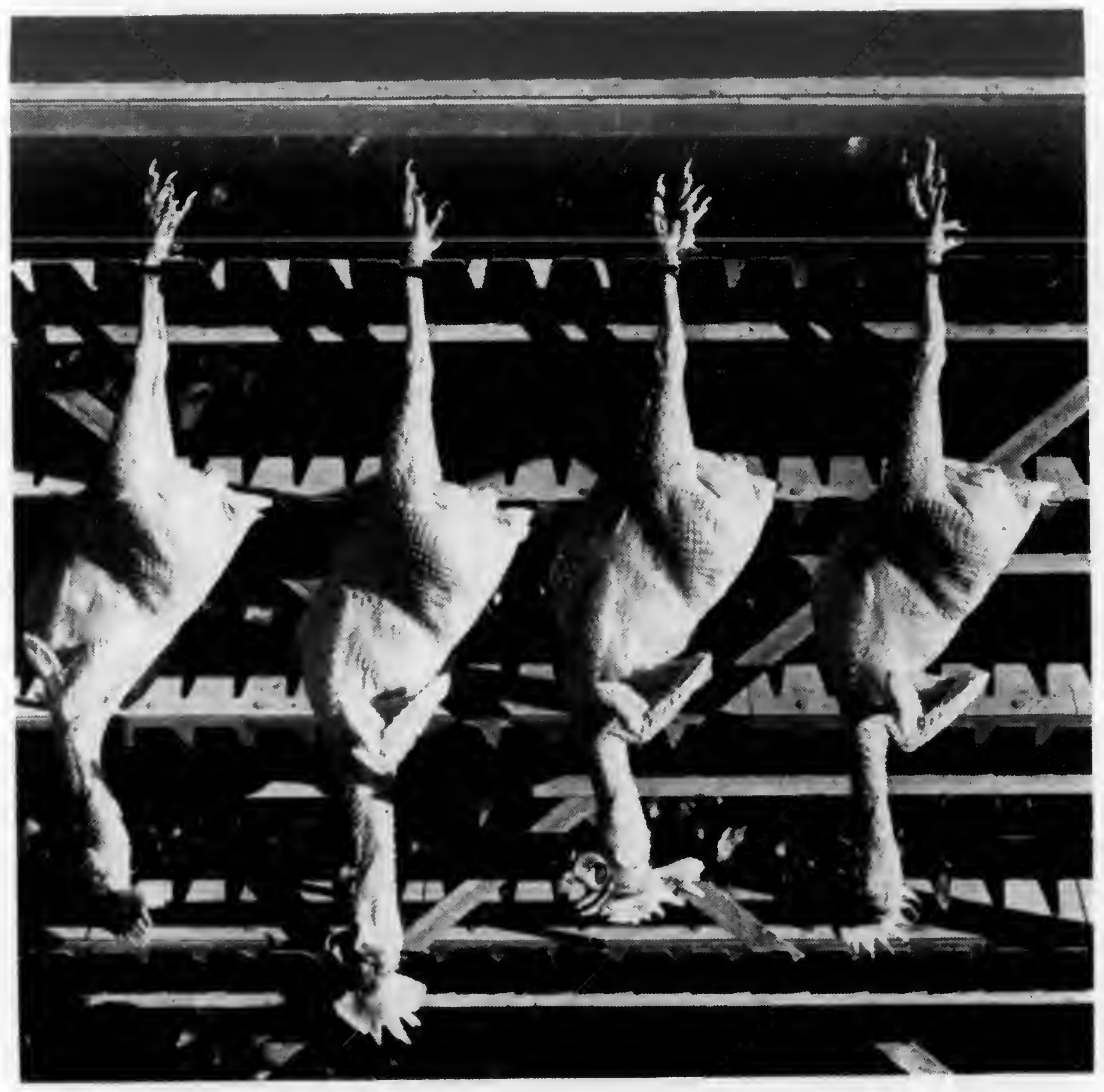
A summary of results obtained during a 52-week laying period is presented in Table 7. Detailed data are given in the appendix. The same method of procedure was used in the collection of these data as was used in the work with the confined groups. No differences in the appearance or condition of the pullets were observed during the year. The addition of vitamin D from the cod liver oil concentrate to an adequate ration fed to pullets having access to range throughout the laying year did not produce any measurable differences in maintenance of body weight, per cent egg production, per cent and quality of egg shell, blood serum calcium, and hatchability of fertile eggs.

TABLE 7—SUMMARY OF RESULTS WITH LAYING PULLETS *
RANGE GROUPS, 1934-35 EXPERIMENT

Group number	10	11	12	13
U.S.P. units vitamin D per 100 grams feed	0	19	39	78
Body weight in pounds per pullet	3.94	3.96	3.97	3.91
Per cent egg production	50.6%	48.7%	52.7%	45.2%
Number of eggs per pullet	184	177	192	165
Weight of eggs in grams †	55.9	54.9	54.9	53.2
Per cent shell of egg	9.1%	9.1%	9.1%	9.1%
Per cent ash in egg shell	53.3%	53.3%	53.4%	53.3%
Milligrams calcium per 100 cc serum	26.6	27.1	26.8	27.7
Per cent hatch of fertile eggs	84.1%	88.0%	85.8%	83.8%

* The data given were taken from Tables 28, 30, 31, 32 and 33 of the appendix and are averages for 52 weeks of egg production.
† A 2-ounce egg weighs 56.7 grams.

With climatic conditions similar to those under which this experiment was conducted during the years of 1934 and 1935 it is possible for laying pullets to secure sufficient irradiation from direct sunlight to adequately meet their vitamin D requirements for satisfactory egg production, maintenance of body weight, and reproduction. In order for pullets to secure enough vitamin D through irradiation from direct sunlight for satisfactory performance they should not be confined at any time during the laying year.



Group number	10	11	12	13
U. S. P. units vitamin D per 100 grams feed	0	19	39	78

FIG. 8.—CONDITION OF BREAST BONE OF PULLETS AT 76 WEEKS OF AGE RANGE GROUPS, 1934-35 EXPERIMENT

TABLE 8—CONDITION OF BREAST BONE OF PULLETS AT 76 WEEKS OF AGE RANGE GROUPS, 1934–35 EXPERIMENT

Group number	10	11	12	13
U.S.P. units vitamin D per 100 grams feed	0	19	39	78
	Per cent deformity			
Very deformed	10.3	5.7	9.1	0.0
Deformed	2.6	5.7	9.1	15.8
Slightly deformed	30.7	34.3	30.3	44.7
Straight	56.4	54.3	51.5	39.5
	Per cent ash in tibia			
Average of 3 pullets	61.2	60.2	62.7	63.7

Effect of Vitamin D on Breast Bone Conformation—The four range groups of pullets were carefully examined at 76 weeks of age for deformed breast bones. The breast bone of each pullet was classified according to four arbitrary degrees of deformity: very deformed, deformed, slightly deformed, and straight. The per cent of the pullets with breast bones showing the various degrees of deformity is given in Table 8. Figure 8 shows the condition of the breast bone of a representative pullet from each group. There was no significant difference in the condition of the breast bones among the four range groups of pullets at 76 weeks of age.

Number of U. S. P. Units of Vitamin D Required For Various Quantities of Feed

In an all-mash ration fed to growing chicks reared in total confinement 39 U. S. P. units of vitamin D per 100 grams of feed is recommended provided the calcium and phosphorus content of the ration is adequate. Since there are approximately 454 grams in a pound, one pound of the mash of an all-mash ration should contain 177 U. S. P. units of vitamin D or 17,700 U. S. P. units in each 100 pounds of mash. When equal parts of mash and scratch grain are fed, 100 grams of mash should contain 78 U. S. P. units of vitamin D. This is equivalent to 354 U. S. P. units per pound or 35,400 U. S. P. units per 100 pounds of mash.

To meet the vitamin D requirements of laying pullets maintained in total confinement 100 grams of an all-mash ration should contain 78 U. S. P. units of vitamin D. One pound of the mash should contain 354 U. S. P. units and 100 pounds 35,400 U. S. P. units of vitamin D. When confined laying pullets are fed equal parts of mash and scratch grain, 100 grams of mash should contain 156 U. S. P. units of vitamin D. This is equivalent to 708 U. S. P. units per pound or 70,800 U. S. P. units per 100 pounds of mash.

TABLE 9—QUANTITIES OF OILS OF VARIOUS POTENCIES REQUIRED TO SUPPLY OPTIMUM AMOUNTS OF VITAMIN D IN GROWING AND LAYING RATIONS

Vitamin D potency [1] of oil (U.S.P. units per gram)	No. of U.S.P. [2] units per pound of oil	No. of U.S.P. [3] units required in 100 pounds of mash	Approximate per cent [4] of oil to be added to the mash	Approximate pounds of oil to be added to one ton of mash
When an all-mash ration is fed to growing chicks:				
50	22,700	17,700	0.78 or ¾	15
85	38,590	17,700	0.46 or ½	10
310	140,740	17,700	0.13 or ⅛	2½
400	181,600	17,700	0.10 or 1/10	2
When a grain and mash ration (equal parts) is fed to growing chicks or an all-mash ration is fed to laying hens:				
50	22,700	35,400	1.56 or 1½	30
85	38,590	35,400	0.92 or 1	20
310	140,740	35,400	0.25 or ¼	5
400	181,600	35,400	0.19 or ⅕	4
When a grain and mash ration (equal parts) is fed to laying and breeding hens:				
50	22,700	70,800	3.12 or 3	60
85	38,590	70,800	1.83 or 2	40
310	140,740	70,800	0.50 or ½	10
400	181,600	70,800	0.39 or ⅖	8

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[1] In simple terms a U.S.P. unit is a measuring stick used in expressing the potency of a vitamin D substance in terms of a reference standard.

[2] The number of U.S.P. units per pound of oil is obtained by multiplying the number of U.S.P. units per gram of oil by 454 (the number of grams is one pound).

[3] These recommendations are the results of an extensive four-year study on vitamin D requirements of chickens.

[4] The approximate per cent of oil to be added to the mash is obtained by dividing the number of U.S.P. units required per 100 lbs. of mash by the number of U.S.P. units per pound of oil.

The potency of oils used as sources of vitamin D for poultry feeding vary considerably. This variation in potency frequently causes confusion concerning the amount of an oil of a particular potency to add to the ration. Table 9 shows the method of computing the amount of an oil of known potency necessary to add to the mash to fulfill the requirements of growing chicks and laying hens for vitamin D. The table is based on results which have been gathered over a period of four years and which have involved a total of over 6,000 chicks and 1,050 laying hens.

General Summary

1. Single Comb White Leghorn chicks, fed an all-mash ration deficient in vitamin D but adequate in all other known food essentials, and reared in battery brooders or on the floor and deprived of sunshine, developed rickets at approximately three and one-half weeks of age.
2. For chicks deprived of sunshine from hatching time to 24 weeks of age, the minimum protective level of vitamin D was 19 U. S. P. units per 100 grams of feed.
3. Very satisfactory results in growth and bone development were obtained in both experiments when 39 U. S. P. units of vitamin D per 100 grams of feed from cod liver oil concentrate in cod liver oil were supplied to Single Comb White Leghorn chicks.
4. With climatic conditions similar to those under which these studies were conducted Single Comb White Leghorn chicks hatched during the month of April and given access to range during the growing period may secure sufficient vitamin D by irradiation from direct sunshine for satisfactory growth and normal bone development.
5. Single Comb White Leghorn laying pullets confined without access to sunshine gave unsatisfactory results when fed an all-mash ration which contained 58 U. S. P. units or less of vitamin D per 100 grams of feed.
6. Single Comb White Leghorn pullets confined without access to sunshine and fed 78 U. S. P. units of vitamin D per 100 grams of feed from time of hatch gave satisfactory results in the maintenance of body weight, egg production, size of egg, quality of egg shell, and hatchability.
7. With climatic conditions similar to those under which these studies were conducted Single Comb White Leghorn Pullets given access to range throughout the entire laying period may secure sufficient vitamin D by irradiation from direct sunshine for satisfactory performance.

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APPENDIX

TABLE 10—AVERAGE BODY WEIGHT OF PULLETS IN POUNDS
1932-33 EXPERIMENT

Group number	1	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	0	10	19	39	58	78	116	155	0 (Range)	39 (Range)
					Growing period					
Age in weeks										
0	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.08	0.07
2	0.21	0.22	0.23	0.22	0.21	0.23	0.21	0.22	0.20	0.21
4	0.37	0.44	0.48	0.48	0.45	0.48	0.46	0.47	0.45	0.47
6	0.55	0.71	0.80	0.84	0.78	0.80	0.77	0.79	0.79	0.83
8	0.66	0.98	1.19	1.23	1.19	1.22	1.16	1.18	1.18	1.24
10	0.72	1.18	1.54	1.58	1.56	1.62	1.53	1.51	1.63	1.62
12	0.81	1.32	1.83	1.84	1.84	1.95	1.81	1.85	2.03	1.97
14	1.21	1.75	2.30	2.32	2.33	2.30	2.22	2.13	2.41	2.37
16	1.34	1.92	2.49	2.50	2.59	2.33	2.46	2.33	2.61	2.61
20	*	2.20	2.96	2.94	2.99	2.95	2.99	2.92	3.02	3.12
24	--------	2.57	3.35	3.40	3.37	3.38	3.42	3.32	3.54	3.62
					Laying period					
28	--------	2.88	3.64	3.61	3.77	3.75	3.80	3.64	3.80	3.78
32	--------	3.04	3.61	3.52	3.72	3.64	3.78	3.55	3.80	3.82
36	--------	3.05	3.53	3.47	3.64	3.66	3.71	3.45	3.82	3.68
40	--------	3.36	4.01	3.92	4.03	3.88	4.10	3.90	4.10	3.94
44	--------	3.12	3.82	3.73	3.81	3.89	3.90	3.81	3.88	3.88
48	--------	3.08	3.80	3.49	3.63	3.73	3.74	3.62	3.80	3.82
52	--------	3.15	3.70	3.50	3.66	3.71	3.67	3.64	3.88	3.87
56	--------	2.97	3.47	3.55	3.61	3.81	3.73	3.60	3.78	3.83
60	--------	2.87	3.36	3.39	3.55	3.67	3.68	3.49	3.61	3.62
64	--------	2.76	3.37	3.46	3.49	3.55	3.62	3.45	3.58	3.64
68	--------	2.84	3.29	3.41	3.42	3.51	3.52	3.39	3.51	3.70
72	--------	3.08	3.31	3.49	3.44	3.59	3.58	3.41	3.55	3.75
76	--------	3.09	3.43	3.50	3.51	3.53	3.50	3.39	3.60	3.77
Average 28 to 76 weeks	--------	3.02	3.56	3.54	3.64	3.69	3.72	3.56	3.75	3.78

* Group 1 was discontinued at 16 weeks.

TABLE 11—AVERAGE BODY WEIGHT OF PULLETS IN POUNDS
1934-35 EXPERIMENT

Group number	1	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	0	19	39	58	78	97	116	155	310
					Growing period				
Age in weeks									
0	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
2	0.17	0.17	0.17	0.16	0.14	0.16	0.17	0.16	0.14
4	0.43	0.46	0.47	0.45	0.44	0.44	0.45	0.43	0.38
6	0.55	0.79	0.83	0.81	0.80	0.81	0.82	0.79	0.77
8	0.65	1.14	1.22	1.23	1.19	1.25	1.25	1.26	1.23
10	*	1.51	1.61	1.64	1.55	1.64	1.63	1.60	1.62
12	--------	1.84	2.05	2.07	1.97	2.02	2.05	1.99	2.00
14	--------	2.17	2.31	2.30	2.21	2.26	2.31	2.28	2.30
16	--------	2.62	2.76	2.76	2.63	2.65	2.73	2.69	2.65
20	--------	3.33	3.42	3.45	3.26	3.37	3.42	3.36	3.42
24	--------	3.40	3.53	3.59	3.42	3.53	3.65	3.60	3.65
					Laying period				
28	--------	3.15	3.13	3.19	3.06	3.21	3.30	3.15	3.35
32	--------	3.20	3.34	3.33	3.30	3.39	3.57	3.43	3.61
36	--------	3.23	3.31	3.38	3.31	3.31	3.55	3.50	3.58
40	--------	3.13	3.29	3.40	3.28	3.39	3.70	3.63	3.65
44	--------	3.07	3.31	3.52	3.36	3.34	3.77	3.61	3.79
48	--------	3.48	3.60	3.74	3.68	3.62	3.86	3.82	4.06
52	--------	3.75	3.75	3.92	3.85	3.83	4.06	4.08	4.22
56	--------	3.69	3.76	3.85	3.87	3.89	4.10	4.09	4.25
60	--------	3.56	3.68	3.76	3.79	3.80	3.97	3.99	4.10
64	--------	3.71	3.71	3.86	3.80	3.78	3.99	3.93	4.14
68	--------	3.59	3.64	3.87	3.90	3.87	4.04	3.99	4.15
72	--------	3.52	3.71	3.81	3.80	3.86	4.01	4.01	4.15
Average 28 to 72 weeks	--------	3.42	3.52	3.64	3.58	3.61	3.83	3.77	3.92

* Group 1 was discontinued at eight weeks.

TABLE 12—FEED CONSUMPTION

(Average number of pounds of mash consumed per pullet from 0 to 76 weeks)
1932–33 Experiment

Group number	1	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	0	16	19	39	58	78	116	155	0 (Range)	39 (Range)
Growing period										
Weeks										
0–2	0.34	0.36	0.36	0.36	0.33	0.37	0.36	0.34	0.45	0.48
2–4	0.63	0.72	0.76	0.79	0.71	0.73	0.74	0.77	0.73	0.79
4–6	0.76	0.88	1.14	1.18	1.12	1.13	1.07	1.09	1.17	1.16
6–8	0.85	1.12	1.37	1.37	1.39	1.38	1.32	1.36	1.41	1.54
8–10	0.79	1.29	1.68	1.69	1.71	1.82	1.66	1.62	1.78	1.94
10–12	0.84	1.23	1.73	1.79	1.89	1.87	1.75	1.88	2.15	2.45
12–14	0.53	0.74	1.27	1.26	1.23	1.09	1.07	0.93	1.19	1.22
14–16	1.52	1.62	1.79	1.91	2.00	1.79	1.93	2.04	2.07	1.72
16–20	*	3.12	4.35	4.29	4.37	4.61	4.42	4.91	4.18	4.92
20–24	--------	3.94	5.01	5.03	4.90	4.85	4.96	5.09	4.84	5.26
Total number pounds consumed	--------	15.01	19.46	19.67	19.65	19.64	19.28	20.03	19.97	21.48
Laying period										
Weeks										
24–28	--------	4.22	4.61	5.49	5.92	5.87	5.73	5.67	5.60	5.85
28–32	--------	4.65	5.11	5.70	5.96	6.12	6.08	5.79	5.81	5.94
32–36	--------	4.39	4.59	5.27	5.23	5.98	5.92	5.69	6.01	5.65
36–40	--------	4.20	4.82	5.65	6.59	6.65	6.78	6.94	6.82	6.55
40–44	--------	5.17	5.93	6.73	7.26	7.08	6.90	7.57	6.86	7.11
44–48	--------	4.92	5.80	6.42	6.79	6.28	6.38	6.79	6.78	6.21
48–52	--------	5.75	6.11	6.66	6.84	6.67	6.93	6.80	6.93	6.83
52–56	--------	5.33	5.79	6.80	6.85	6.93	7.04	7.19	6.77	6.54
56–60	--------	4.63	5.51	6.43	7.27	6.70	6.83	6.58	6.00	6.02
60–64	--------	3.83	4.61	5.09	5.87	5.80	5.37	5.56	5.14	5.45
64–68	--------	4.23	4.56	5.56	5.71	5.85	5.42	5.69	5.66	5.91
68–72	--------	4.47	4.60	5.29	4.80	5.51	5.25	5.28	5.25	5.80
72–76	--------	4.38	4.46	5.11	5.34	5.84	5.40	5.04	5.34	5.42
Total number pounds consumed	--------	60.17	66.50	76.20	80.43	81.28	80.12	80.59	78.97	79.28

TABLE 13—FEED CONSUMPTION

(Average number of pounds of mash consumed per pullet from 0 to 76 weeks)
1934–35 Experiment

Group number	1	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	0	19	39	58	78	97	116	155	310
Growing period									
Weeks									
0–2	0.41	0.43	0.43	0.45	0.43	0.45	0.42	0.43	0.41
2–4	0.65	0.72	0.73	0.74	0.70	0.70	0.71	0.73	0.73
4–6	0.87	1.21	1.24	1.28	1.22	1.22	1.16	1.21	1.24
6–8	0.81	1.54	1.51	1.55	1.49	1.55	1.59	1.83	1.57
8–10	*	1.67	1.86	1.87	1.78	1.85	1.98	1.92	1.90
10–12	--------	2.08	2.18	2.24	2.13	2.17	2.20	2.20	2.25
12–14	--------	2.10	2.24	2.28	2.17	2.20	2.21	2.30	2.34
14–16	--------	2.19	2.48	2.47	2.35	2.35	2.31	2.38	2.30
16–20	--------	6.49	6.80	6.82	6.50	6.96	6.72	6.77	6.63
20–24	--------	5.77	6.11	6.34	5.96	6.41	6.44	6.65	6.38
Total number pounds consumed	--------	24.20	25.58	26.04	24.73	25.86	25.74	26.42	25.75
Laying period									
Weeks									
24–28	--------	4.43	4.66	4.87	5.13	5.01	5.55	5.17	5.34
28–32	--------	4.05	4.52	4.95	5.65	5.44	5.54	5.59	5.57
32–36	--------	4.49	4.37	4.52	4.56	5.04	5.37	5.79	5.69
36–40	--------	4.47	4.45	4.83	5.28	5.39	5.96	6.59	5.99
40–44	--------	4.45	4.55	5.32	5.43	5.42	6.41	5.88	6.11
44–48	--------	5.50	5.33	6.39	6.37	6.08	6.44	6.34	6.82
48–52	--------	6.04	5.76	6.72	7.20	6.87	6.76	7.15	7.41
52–56	--------	5.94	5.99	6.93	7.25	7.31	7.21	7.50	7.68
56–60	--------	5.63	5.73	6.73	6.73	6.99	7.09	7.17	7.15
60–64	--------	6.07	6.64	7.19	7.16	6.81	7.21	7.37	7.58
64–68	--------	6.11	6.59	7.19	7.50	7.34	7.32	7.21	7.63
68–72	--------	5.12	5.53	5.96	6.44	6.33	6.60	6.20	7.16
Total number pounds consumed	--------	62.30	64.12	71.60	74.70	74.03	77.46	77.96	80.13

* Group 1 was discontinued at eight weeks.

TABLE 14—VITAMIN D INTAKE

(Average number of U.S.P. units from cod liver oil concentrate consumed per pullet)
1932–33 Experiment

Group number	1	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	0	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Growing period										
Weeks										
0–2	0	16	32	63	88	128	188	240	0	84
2–4	0	32	67	138	186	258	391	539	0	138
4–6	0	39	100	207	296	397	565	763	0	204
6–8	0	49	121	241	366	484	697	955	0	271
8–10	0	57	148	297	452	641	877	1,184	0	341
10–12	0	54	152	315	499	658	922	1,320	0	430
12–14	0	33	112	222	325	381	565	653	0	214
14–16	0	71	157	336	527	629	1,018	1,435	0	302
16–20	0	137	382	754	1,153	1,620	2,331	3,452	0	865
20–24	0	173	441	884	1,291	1,706	2,617	3,578	0	925
Total number units consumed	0	661	1,712	3,457	5,183	6,902	10,171	14,119	0	3,774
Laying period										
24–28	0	186	405	965	1,560	2,064	3,619	3,983	0	1,029
28–32	0	204	449	1,001	1,572	2,152	3,204	4,072	0	1,043
32–36	0	193	403	926	1,378	2,102	3,119	4,000	0	993
36–40	0	185	423	993	1,738	2,336	3,575	4,882	0	1,151
40–44	0	227	521	1,182	1,915	2,488	3,684	5,324	0	1,250
44–48	0	216	510	1,129	1,791	2,209	3,365	4,772	0	1,092
48–52	0	253	537	1,171	1,803	2,345	3,653	4,778	0	1,201
52–56	0	234	509	1,195	1,807	2,437	3,712	5,055	0	1,150
56–60	0	203	484	1,131	1,916	2,356	3,603	4,624	0	1,058
60–64	0	168	405	895	1,548	2,042	2,834	3,908	0	959
64–68	0	186	400	977	1,504	2,056	2,858	4,000	0	1,039
68–72	0	196	494	930	1,267	1,938	2,768	3,711	0	1,020
72–76	0	192	392	899	1,407	2,053	2,845	3,541	0	953
Total number units consumed	0	2,643	5,842	13.394	21,206	28,578	42,239	56,650	0	13.938

TABLE 15—VITAMIN D INTAKE

(Average number of U.S.P. units from cod liver oil concentrate consumed per pullet)
1934–35 Experiment

Group number	1	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	0	19	39	58	78	97	116	155	310
Growing period									
Weeks									
0–2	0	38	76	117	150	196	219	302	570
2–4	0	63	128	194	245	305	376	512	1,032
4–6	0	106	218	238	429	536	610	853	1,738
6–8	0	135	265	408	523	683	836	1,287	2,210
8–10	0	147	328	492	625	811	1,042	1,348	2,664
10–12	0	182	383	590	748	954	1,158	1,545	3,160
12–14	0	185	394	601	762	968	1,163	1,616	3,290
14–16	0	192	435	650	825	1,034	1,216	1,673	3,234
16–20	0	570	1,195	1,799	2,284	3,059	3,541	4,762	9,319
20–24	0	507	1,073	1,671	2,095	2,817	3,395	4,672	8,972
Total number units consumed	0	2,125	4,495	6,860	8,686	11,363	13,556	18,570	36,189
Laying period									
Weeks									
24–28	0	389	819	1,283	1,802	2,202	2,926	3,635	7,509
28–32	0	356	794	1,304	1,986	2,388	2,920	3,933	7,827
32–36	0	395	768	1,191	1,604	2,214	2,832	4,068	8,007
36–40	0	393	781	1,274	1,857	2,367	3,144	4,631	8,417
40–44	0	391	800	1,402	1,910	2,380	3,381	4,133	8,584
44–48	0	483	936	1,686	2,238	2,671	3,398	4,455	9,589
48–52	0	531	1,012	1,771	2,532	3,019	3,562	5,026	10.415
52–56	0	522	1,053	1,826	2,549	3,210	3,803	5,275	10,801
56–60	0	495	1,008	1,775	2,367	3,071	3,738	5,044	10,048
60–64	0	533	1,167	1,895	2,517	2,994	3,803	5,181	10,653
64–68	0	537	1,157	1,897	2,637	3,224	3,862	5,066	10,725
68–72	0	450	973	1,572	2,264	2,781	3,480	4,357	10,064
Total number units consumed	--------	5,485	11,268	18,876	26,263	32,521	40,849	54,804	112,639

TABLE 16—AVERAGE PER CENT EGG PRODUCTION
1932–33 EXPERIMENT

Group number	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Weeks									
24–28	5.3	14.1	40.4	36.9	34.4	40.9	31.2	35.9	49.5
28–32	5.1	23.8	43.6	44.6	57.3	53.2	47.1	47.6	51.5
32–36	1.5	6.8	19.1	19.2	43.0	37.8	25.8	38.1	30.2
36–40	3.2	8.9	30.0	24.4	37.8	35.4	26.6	36.1	24.1
40–44	6.6	23.0	59.3	62.2	59.1	65.0	67.3	57.1	55.7
44–48	10.4	23.4	42.7	55.7	55.2	58.6	54.0	54.8	48.5
48–52	24.0	25.4	47.6	41.7	40.9	54.8	46.0	54.8	54.7
52–56	21.7	27.2	53.9	47.4	58.2	59.2	63.5	61.8	61.2
56–60	8.3	16.7	54.1	54.8	60.3	64.9	62.6	56.0	58.4
60–64	7.6	12.7	35.9	38.3	47.5	43.7	47.0	39.2	38.2
64–68	3.1	11.4	33.9	34.6	45.2	45.4	51.1	33.3	40.9
68–72	0.8	10.3	28.6	16.1	40.2	41.3	40.7	29.7	43.0
72–76	8.0	5.6	23.0	14.4	33.5	30.5	24.5	13.5	32.1
Average	7.6	16.6	39.9	38.6	47.4	48.8	44.7	44.0	45.4

TABLE 17—AVERAGE PER CENT EGG PRODUCTION
1934–35 EXPERIMENT

Group number	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	19	39	58	78	97	116	155	310
Weeks								
20–24	31.8	37.5	47.3	42.4	36.2	41.1	46.7	37.6
24–28	22.0	25.9	39.5	46.5	47.9	54.6	49.8	57.4
28–32	4.9	7.4	20.0	27.9	34.9	43.2	44.3	42.3
32–36	5.9	8.4	10.4	29.5	39.3	49.7	58.2	57.6
36–40	4.7	4.3	12.7	24.7	26.0	43.0	58.2	46.8
40–44	1.1	2.2	9.5	19.1	25.1	41.4	42.4	40.2
44–48	3.2	5.2	18.1	29.1	25.8	37.5	40.7	46.1
48–52	7.3	11.7	26.0	44.0	39.5	37.9	48.0	55.8
52–56	8.6	15.0	29.6	45.6	52.0	49.4	60.2	58.9
56–60	7.9	12.8	26.2	38.2	50.4	50.3	53.1	55.9
60–64	6.2	15.2	24.1	32.1	31.6	42.1	44.6	45.6
64–68	10.5	20.5	26.6	39.5	35.8	35.8	39.9	47.0
68–72	3.7	11.8	22.2	38.1	35.7	34.0	44.5	44.0
Average	10.2	14.5	24.7	35.3	37.0	43.4	48.6	48.8

TABLE 18—AVERAGE WEIGHT OF EGGS IN GRAMS *
1932–33 EXPERIMENT

Group number	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Weeks									
24–28	43.8	43.5	44.8	45.0	45.9	45.5	45.6	45.8	45.7
28–32	46.2	46.4	47.3	47.6	48.1	48.2	47.5	48.8	48.8
32–36	40.9	48.0	49.1	48.9	49.9	50.6	50.0	50.6	50.2
36–40	46.9	49.5	52.2	52.3	52.9	53.6	51.5	53.0	53.3
40–44	51.1	52.1	54.6	54.4	53.9	55.6	54.5	54.1	54.2
44–48	50.8	50.9	52.9	54.5	54.1	53.8	53.9	55.0	54.1
48–52	54.9	53.5	53.8	54.8	55.0	54.9	54.5	55.6	55.6
52–56	54.6	53.8	54.1	55.4	55.3	54.5	54.2	55.0	55.4
56–60	49.6	53.1	53.8	54.7	54.8	54.3	53.5	54.9	54.5
60–64	47.6	52.6	51.8	53.6	53.2	51.9	52.4	53.0	53.7
64–68	50.0	51.8	52.6	54.4	53.8	53.6	53.5	54.3	54.9
68–72	56.7	54.8	53.6	55.6	54.9	55.2	54.5	56.0	55.3
72–76	53.3	58.6	55.2	57.5	56.2	56.8	55.2	56.3	56.6
Average	51.0	50.9	51.7	52.7	52.3	52.7	52.3	53.1	52.9

* A 2-ounce egg weighs 56.7 grams.

TABLE 19—AVERAGE WEIGHT OF EGGS IN GRAMS *
1934–35 EXPERIMENT

Group number	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	19	39	58	78	97	116	155	310
Weeks								
20–24	38.5	40.4	39.9	39.0	40.4	40.6	41.3	40.4
24–28	39.8	41.9	42.5	41.9	42.5	42.7	43.2	43.2
28–32	41.2	41.6	45.1	44.6	45.9	44.2	45.6	44.6
32–36	42.7	44.1	43.6	45.3	46.9	46.4	48.1	47.9
36–40	43.6	43.0	46.2	48.5	48.8	49.4	49.7	48.7
40–44	45.8	48.0	48.5	51.1	51.0	52.5	51.0	50.9
44–48	47.7	49.7	51.5	53.4	54.1	53.4	53.1	53.0
48–52	50.9	53.1	54.8	55.4	56.5	55.3	55.0	54.2
52–56	50.8	53.5	54.0	55.3	57.4	56.0	56.2	55.3
56–60	51.3	52.9	55.1	56.1	57.1	56.3	57.3	55.7
60–64	50.1	54.5	55.8	56.1	57.0	57.0	57.5	55.9
64–68	52.9	56.4	58.1	56.6	58.2	57.1	58.5	56.4
68–72	53.3	57.3	57.5	57.5	57.7	57.8	58.9	59.0
Average	43.1	46.2	48.4	49.8	50.7	50.5	51.2	51.0

* A 2-ounce egg weighs 56.7 grams.

TABLE 20—AVERAGE PER CENT EGG SHELL OF TOTAL WEIGHT OF EGG
1932–33 EXPERIMENT

Group number	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Weeks									
28–32	6.4	7.8	9.7	9.7	10.0	9.7	9.9	10.1	10.1
32–36	6.7	7.6	9.5	9.5	9.4	9.9	10.2	9.8	10.0
36–40	7.1	7.4	9.9	9.3	9.5	9.8	8.9	9.4	8.9
40–44	6.1	7.7	9.1	9.3	9.5	9.8	9.8	9.3	9.6
44–48	6.3	6.6	8.1	8.9	8.8	8.9	9.6	8.9	8.8
48–52	7.9	7.0	9.0	9.2	8.9	9.1	9.2	8.9	8.6
52–56	7.1	7.2	9.2	9.0	8.8	9.0	8.9	9.0	9.0
56–60	6.2	6.9	8.5	8.8	7.7	7.8	8.6	8.6	8.5
60–64	*	6.8	7.9	8.7	8.2	8.7	8.8	8.3	8.6
64–68	*	6.6	7.6	7.9	8.4	7.9	8.8	8.9	8.6
68–72	*	6.2	7.3	8.4	8.3	8.5	9.1	8.7	8.7
72–76	6.6	6.9	8.8	8.9	8.3	8.6	9.2	9.0	8.7
Average	6.7	7.0	8.7	8.9	8.8	9.0	9.2	9.1	9.0

* At the time this determination was made a sufficient number of eggs was not obtainable from Group 2 for this purpose.

TABLE 21—AVERAGE PER CENT EGG SHELL OF TOTAL WEIGHT OF EGG
1934–35 EXPERIMENT

Group number	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	19	39	58	78	97	116	155	310
Weeks								
20–24	7.4	9.2	9.2	10.0	9.6	9.4	9.5	9.7
24–28	5.3	6.6	8.6	7.9	8.3	9.0	9.4	9.1
28–32	5.1	6.3	7.2	7.9	8.6	8.1	8.2	8.0
32–36	5.5	6.3	7.2	7.6	8.3	8.4	9.1	8.9
36–40	5.5	7.1	6.8	8.3	8.2	8.2	8.7	9.0
40–44	5.7	6.3	8.2	8.8	9.2	8.7	9.2	8.8
44–48	6.6	6.5	6.3	8.9	9.5	9.1	8.9	9.1
48–52	6.0	6.9	7.8	8.2	9.1	8.6	9.1	9.5
52–56	6.7	7.5	8.2	8.6	9.0	8.9	8.9	9.8
56–60	6.2	7.4	9.0	8.4	8.7	9.1	9.6	9.3
60–64	6.1	8.0	8.9	8.7	8.7	8.7	9.1	9.5
64–68	6.9	8.6	8.7	8.6	9.0	8.7	9.2	9.3
68–72	6.9	7.4	8.6	8.5	8.9	9.0	8.9	9.1
Average	6.1	7.3	8.1	8.5	8.9	8.8	9.1	9.2

TABLE 22—PER CENT ASH IN EGG SHELL
1932–33 EXPERIMENT

Group number	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Weeks									
28–32	51.8	52.7	52.8	53.4	52.9	53.1	52.5	53.0	52.7
32–36	52.5	52.6	53.2	53.1	53.4	53.0	53.3	53.2	52.7
36–40	52.1	52.5	53.4	53.3	53.2	53.0	53.2	53.2	53.2
40–44	52.3	52.8	53.4	53.4	53.7	54.1	53.4	53.3	53.5
44–48	51.6	52.4	52.9	53.4	53.1	53.1	53.7	53.3	53.0
48–52	52.5	52.4	53.0	53.0	52.9	53.3	53.2	53.3	53.1
52–56	51.9	52.2	52.9	53.4	53.1	53.2	52.7	53.3	53.3
56–60	51.7	52.3	53.0	53.4	53.2	53.2	53.3	53.3	53.1
60–64	*	51.7	52.9	53.3	53.2	53.3	53.5	53.2	53.0
64–68	*	52.3	52.9	53.1	53.3	53.2	53.3	53.5	53.3
68–72	*	51.7	52.4	53.2	53.3	53.0	53.3	52.8	53.3
72–76	52.6	52.6	53.2	53.5	53.1	53.1	53.1	53.2	53.4
Average	52.1	52.4	53.0	53.3	53.2	53.2	53.2	53.2	53.1

* At the time this determination was made a sufficient number of eggs was not obtainable from Group 2 for this purpose.

TABLE 23—PER CENT ASH IN EGG SHELL
1934–35 EXPERIMENT

Group number	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	19	39	58	78	97	116	155	310
Weeks								
20–24	53.1	53.3	53.5	53.7	53.7	53.5	53.5	53.5
24–28	50.8	51.8	52.7	52.9	53.3	53.7	53.4	53.3
28–32	51.2	52.2	52.8	52.9	52.9	52.8	53.0	52.9
32–36	51.5	52.0	52.3	52.9	53.1	53.6	53.4	53.2
36–40	51.4	52.7	52.4	53.4	53.5	53.7	53.1	53.3
40–44	50.3	52.2	53.2	53.3	53.6	53.4	53.5	53.3
44–48	52.1	52.4	53.0	53.3	53.6	53.6	53.3	53.3
48–52	51.9	52.2	53.0	53.2	53.4	53.3	53.1	52.6
52–56	52.3	52.8	52.8	53.2	53.3	53.4	53.4	53.5
56–60	51.7	52.8	53.2	53.0	53.4	53.6	53.6	53.5
60–64	51.8	53.0	53.4	53.4	53.5	53.4	53.5	53.5
64–68	52.0	53.3	53.4	53.5	53.5	53.5	53.7	53.4
68–72	52.6	52.6	53.5	53.2	53.1	53.7	53.4	53.1
Average	51.7	52.6	53.0	53.2	53.4	53.5	53.4	53.3

TABLE 24—BLOOD SERUM CALCIUM
(Milligrams calcium per 100 cubic centimeters of blood serum)
1932–33 Experiment

Group number	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed	10	19	39	58	78	116	155	0 (Range)	39 (Range)
Weeks									
28–32	19.0	18.1	20.4	20.4	22.5	24.4	25.1	27.3	22.6
32–36	17.4	21.4	25.2	26.2	21.9	24.6	23.5	22.6	22.6
36–40	17.1	21.3	25.8	27.0	25.1	27.4	30.3	25.5	25.3
40–44	22.3	22.8	22.6	23.3	23.4	25.4	23.2	27.7	27.6
44–48	20.3	22.3	26.6	28.5	27.6	29.8	30.0	28.0	27.4
48–52	21.0	20.6	27.6	26.5	27.2	28.0	27.4	30.3	28.8
52–56	15.2	21.8	27.4	28.6	29.0	29.6	28.7	26.1	28.9
56–60	21.1	19.9	26.5	24.0	24.6	25.0	20.2	27.4	25.8
60–64	14.6	27.6	21.9	23.9	26.6	25.0	22.7	28.3	22.4
64–68	*	18.8	21.0	20.8	25.2	25.7	27.3	28.0	27.0
68–72	*	17.0	19.8	22.6	26.1	23.4	19.0	20.9	21.8
72–76	18.6	25.1	26.6	24.8	27.0	23.8	25.4	27.5	25.6
Average	18.7	21.4	24.3	24.7	25.5	26.0	25.2	26.6	25.5

* At the time this determination was made sufficient pullets were not in production from which to obtain a sample of blood.

TABLE 25—BLOOD SERUM CALCIUM
(Milligrams calcium per 100 cubic centimeters of blood serum)
1934–35 Experiment

Group number	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed	19	39	58	78	97	116	155	310
Weeks								
20–24	24.6	25.1	25.1	27.2	26.7	26.9	30.0	27.0
24–28	23.7	29.7	24.9	25.7	21.9	27.4	28.0	30.4
28–32	18.0	19.2	21.5	21.5	23.8	22.3	22.3	29.4
32–36	19.5	20.3	21.3	20.2	27.4	23.6	24.3	29.5
36–40	22.4	12.9	15.2	19.4	25.0	22.9	24.0	23.4
40–44	20.2	18.2	25.5	22.5	23.6	23.3	24.2	24.7
44–48	21.7	20.1	24.7	24.4	24.6	26.1	25.3	26.4
48–52	15.8	18.3	23.4	24.6	27.2	25.0	29.6	30.4
52–56	16.6	24.3	19.0	30.1	28.2	25.5	29.8	30.0
56–60	18.5	20.3	22.5	20.7	26.1	25.1	28.3	26.6
60–64	26.8	27.2	28.8	30.3	28.8	29.0	30.1	29.6
64–68	28.0	22.6	25.6	24.1	29.3	27.5	30.3	29.8
68–72	19.9	26.3	25.5	27.3	28.3	28.6	28.3	29.4
Average	21.2	21.9	23.3	24.5	26.2	25.6	27.3	28.2

TABLE 26—HATCHING RESULTS
1932–33 EXPERIMENT

Hatch number	Date hatched	2	3	4	5	6	7	8	9	10
U.S.P. units vitamin D per 100 grams feed		10	19	39	58	78	116	155	0 (Range)	39 (Range)
	Number of eggs set									
1	March 10, 1933	5	46	100	100	100	100	100	100	100
2	April 7, 1933	41	86	100	100	100	100	100	100	100
3	May 12, 1933	61	80	100	100	100	100	100	100	100
4	June 1, 1933	18	34	100	100	100	100	100	100	100
5	June 16, 1933	6	38	100	100	100	100	100	100	100
Total 5 hatches		131	284	500	500	500	500	500	500	500
	Per cent hatch of fertile eggs									
1	March 10, 1933	25.0	40.5	87.8	80.7	89.4	90.1	96.7	87.3	92.0
2	April 7, 1933	69.2	54.9	61.0	81.2	79.3	92.1	80.0	93.8	85.4
3	May 12, 1933	66.1	38.5	70.8	96.1	80.2	92.5	85.3	86.1	76.1
4	June 1, 1933	7.1	41.9	75.7	87.0	90.9	87.0	88.7	90.7	86.2
5	June 16, 1933	0.0	41.4	76.2	84.0	84.5	78.6	90.3	97.4	77.5
Average 5 hatches		56.4	44.5	74.6	84.5	84.9	88.1	87.8	91.0	83.3

TABLE 27—HATCHING RESULTS
1934–35 EXPERIMENT

Hatch number	Date hatched	2	3	4	5	6	7	8	9
U.S.P. units vitamin D per 100 grams feed		19	39	58	78	97	116	155	310
	Number of eggs set								
1	March 6, 1935	14	47	77	100	76	100	100	100
2	April 3, 1935	16	30	72	100	100	100	100	100
3	May 29, 1935	11	34	42	90	71	100	100	100
Total 3 hatches		41	111	191	290	247	300	300	300
	Per cent hatch of fertile eggs								
1	March 6, 1935	25.0	34.2	82.5	77.5	77.3	68.3	77.8	72.6
2	April 3, 1935	00.0	30.4	69.8	75.6	82.7	77.0	91.8	89.0
3	May 29, 1935	10.0	52.4	80.6	80.6	78.2	76.3	89.2	68.8
Average 3 hatches		11.1	37.8	77.4	77.7	79.6	74.2	86.5	77.1

TABLE 28—AVERAGE BODY WEIGHT OF PULLETS IN POUNDS RANGE GROUPS, 1934–35 EXPERIMENT

Group number	10	11	12	13
U.S.P. units vitamin D per 100 grams feed	0	19	39	78
	Growing period			
Age in weeks				
0	0.08	0.08	0.08	0.08
2	0.23	0.24	0.23	0.23
4	0 46	0.50	0.49	0.49
6	0.79	0.85	0.82	0.80
8	1.26	1.22	1.24	1.20
10	1.63	1.62	1.57	1.57
12	2.00	2.06	1.99	1.99
14	2.31	2.34	2.32	2.28
16	2.78	2.83	2.82	2.76
20	3.08	3.16	3.13	3.14
24	3.52	3.59	3.59	3.53
	Laying period			
28	3.81	3.91	3.80	3.81
32	3.89	3.95	3.92	3.95
36	3.98	4.01	3.90	4.00
40	3.83	3.87	3.87	3.87
44	3.66	3.68	3.73	3.71
48	3.91	3.98	3.91	3.95
52	4.00	4.06	4.06	4.02
56	4.03	4.09	4.13	4.07
60	3.92	4.01	4.00	3.89
64	4.01	4.04	4.08	3.86
68	4.07	4.04	4.07	3.98
72	4.07	3.93	4.06	3.92
76	4.10	3.94	4.11	3.93
Average 28 to 76 weeks	3.94	3.96	3.97	3.91

TABLE 29—FEED CONSUMPTION AND VITAMIN D INTAKE

(Average number of pounds of mash and number of U.S.P. units from cod liver oil concentrate consumed per pullet)

Range Groups, 1934–35 Experiment

Group number	10		11		12		13	
U.S.P. units vitamin D per 100 grams feed	0		19		39		78	
	Growing period							
Weeks	Pounds	Units	Pounds	Units	Pounds	Units	Pounds	Units
0–2	0.62	0	0.62	55	0.62	109	0.60	210
2–4	0.82	0	0.84	74	0.84	147	0.80	282
4–6	1.21	0	1.29	113	1.18	207	1.17	410
6–8	1.47	0	1.49	131	1.47	258	1.37	480
8–10	1.88	0	2.03	178	1.72	301	1.72	604
10–12	1.79	0	1.90	167	1.72	302	1.64	576
12–14	2.66	0	2.37	209	2.26	397	2.27	799
14–16	2.18	0	2.65	233	2.34	411	2.61	916
16–20	4.44	0	4.57	402	4.26	748	4.55	1,599
20–24	4.95	0	5.52	485	5.20	914	5.04	1,773
Total pounds or units consumed	21.42	0	23.28	2,047	21.61	3,794	21.77	7,649
	Laying period							
24–28	5.98	0	5.78	508	6.47	1,137	6.15	2,162
28–32	6.14	0	6.30	553	6.70	1,177	6.38	2,243
32–36	6.66	0	6.79	597	6.65	1,168	6.59	2,316
36–40	7.34	0	7.30	642	7.63	1,340	7.14	2,510
40–44	7.33	0	7.20	660	7.76	1,364	7.63	2,682
44–48	6.97	0	6.84	601	7.53	1,323	7.16	2,517
48–52	7.33	0	7.23	636	7.56	1,329	7.20	2,531
52–56	7.21	0	7.09	623	7.78	1,368	7.19	2,529
56–60	6.39	0	6.11	537	6.90	1,212	6.16	2,164
60–64	6.51	0	6.60	580	6.76	1,187	6.15	2,163
64–68	7.68	0	7.61	669	7.78	1,367	6.72	2,364
68–72	6.90	0	6.83	601	7.58	1,332	6.47	2,274
72–76	6.02	0	6.34	557	7.03	1,235	6.35	2,234
Total pounds or units consumed	88.46	0	88.02	7,764	94.13	16,539	87.29	30,689

TABLE 30—AVERAGE PER CENT EGG PRODUCTION AND AVERAGE WEIGHT OF EGGS IN GRAMS* RANGE GROUPS, 1934–35 EXPERIMENTS

Group number	10		11		12		13	
U.S.P. units vitamin D per 100 grams feed	0		19		39		78	
Weeks	Per cent production	Weight in grams	Per cent production	Weight in grams	Per cent production	Weight in grams	Per cent production	Weight in grams
24–28	43.4	47.1	39.9	46.7	48.9	47.0	40.3	46.6
28–32	50.2	50.1	49.9	49.7	57.5	49.5	53.8	48.3
32–36	34.2	52.3	45.9	51.9	38.9	51.7	43.0	50.9
36–40	56.8	55.0	56.6	54.1	56.8	54.4	53.0	52.1
40–44	54.8	55.6	55.1	54.9	56.5	55.7	50.9	53.3
44–48	46.5	56.6	47.5	55.4	49.0	55.7	44.7	53.5
48–52	54.3	58.2	49.2	57.6	51.9	57.1	52.3	55.6
52–56	55.4	58.5	45.5	58.4	56.0	57.5	49.9	56.1
56–60	51.9	57.5	46.8	56.9	53.7	56.7	40.2	55.7
60–64	61.7	57.8	60.6	56.9	60.3	57.4	48.4	55.8
64–68	60.4	58.3	56.9	57.1	62.9	57.8	42.2	54.5
68–72	50.7	59.2	47.3	57.3	50.2	58.1	36.8	55.3
72–76	39.0	60.4	29.6	60.5	43.4	58.6	27.8	57.6
Averages	50.6	55.9	48.7	54.9	52.7	54.9	45.2	53.2

* A 2-ounce egg weighs 56.7 grams.

TABLE 31—AVERAGE PER CENT EGG SHELL AND PER CENT SHELL ASH RANGE GROUPS, 1934–35 EXPERIMENT

Group number	10		11		12		13	
U.S.P. units vitamin D per 100 grams feed	0		19		39		78	
Weeks	Per cent shell	Per cent ash	Per cent shell	Per cent ash	Per cent shell	Per cent ash	Per cent shell	Per cent ash
24–28	10.4	52.9	9.5	53.3	9.6	53.3	10.0	53.2
28–32	9.5	53.4	9.0	53.2	9.8	53.0	9.8	53.5
32–36	9.6	53.3	9.6	53.6	9.4	53.5	9.3	53.4
36–40	9.3	53.6	9.3	53.6	9.2	53.5	9.3	53.6
40–44	9.5	53.4	9.0	53.3	9.3	53.5	9.3	53 5
44–48	9.4	53.5	9.5	53.5	9.1	53.5	9.1	53.6
48–52	9.1	53.2	9.1	53.4	9.6	53.7	9.5	53.3
52–56	9.1	53.4	8.7	53.1	8.8	53.2	8.8	53.1
56–60	8.4	53.4	8.7	53.3	8.8	53.4	8.4	53.0
60–64	8.7	53.2	8.6	53.2	8.9	53.3	8.8	53.4
64–68	8.8	53.5	8.8	53.4	8.6	53.5	8.5	53.4
68–72	9.0	53.3	9.2	53.0	9.0	53.4	8.9	53.3
72–76	8.7	53.3	8.8	53.0	8.8	53.4	8.9	53.2
Averages	9.1	53.3	9.1	53.3	9.1	53.4	9.1	53.3

TABLE 32—BLOOD SERUM CALCIUM

(Milligrams calcium per 100 cubic centimeters of blood serum)

Range Groups, 1934–35 Experiment

Group number	10	11	12	13
U.S.P. units vitamin D per 100 grams feed	0	19	39	78
Weeks				
24–28	27.7	25.9	26.5	28.3
28–32	24.9	25.1	21.8	25.3
32–36	25.4	24.9	27.5	28.3
36–40	24.2	26.8	28.4	29.1
40–44	28.3	30.7	26.4	27.8
44–48	28.6	31.0	28.5	31.1
48–52	27.5	29.9	27.0	30.0
52–56	26.7	24.9	27.7	27.5
56–60	25.6	24.8	27.4	27.5
60–64	29.9	29.5	29.3	28.9
64–68	25.2	25.2	26.8	29.2
68–72	27.1	27.4	24.9	24.6
72–76	24.5	25.7	26.4	22.7
Average	26.6	27.1	26.8	27.7

TABLE 33—HATCHING RESULTS RANGE GROUPS
1934-35 EXPERIMENT

Group number		10	11	12	13
U.S.P. units vitamin D per 100 grams feed		0	19	39	78
		Number of eggs set			
Hatch number	Date hatched				
1	March 6, 1935	100	100	100	100
2	April 3, 1935	100	100	100	100
3	May 29, 1935	100	100	100	100
Total 3 hatches		300	300	300	300
		Per cent hatch of fertile eggs			
1	March 6, 1935	84.1	84.3	88.9	82.8
2	April 3, 1935	88.0	93.0	83.0	85.7
3	May 29, 1935	78.8	85.9	85.9	82.6
Average 3 hatches		84.1	88.0	85.8	83.8

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PROPERTIES OF THE HYDROXYL GROUPS OF CLAY AS A BASIS FOR CHARACTERIZING A MINERAL SOIL[1]

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INTRODUCTION

By the conductometric titration of a soil treated with increasing concentrations of $Ba(OH)_2$, Hissink (17, 18) found that the salt line representing the formation of the barium salt of the soil acids coincided with the abscissa on which were plotted the quantities of $Ba(OH)_2$ added, indicating saturation of these acid functions. It has been found also by Hissink and later by others that the conductivity of the solution at the points of the straight portion of the baryta line is proportional to the content of baryta in solution. Hissink accordingly proposed that the degree of unsaturation of a soil represented by his quantity $T - S$ (T = maximum absorbing capacity, S = replaceable bases) could be determined by the point represented by the intersection of the straight portion of the baryta line with the abscissa. Hissink pointed out that, although the method was not free of objection, the horizontal portion of the bartya line corresponded approximately to the so-called "replaceable" hydrogen ions. But a number of investigators (2, 20, 21, 24, 26) have pointed out that Hissink's quantity ($T - S$) is much higher than that corresponding to the replaceable hydrogen ions.

The resulting confusion appears to have delayed the recognition of the possibilities of the acid and base titrametric method as a means for the rapid characterization of soils. The principal objection which has been raised is that secondary reactions take place—that the barium (or calcium) hydroxide reacts not only with the base exchange components but also with other constituents of the soil—when *an excess* of alkali or alkali hydroxides is added to a soil (21, 25).

On the other hand, by treating soils with increasing concentrations (0.005 N to 0.04 N per gram of soil) of $Ba(OH)_2$ in aqueous solution and also of KOH in alcoholic solution both before and after leaching with a solution of 0.05 N HCl, Clarens (12) has *established the existence of a gamut of acidic functions in a soil, the barium salts of one of which are decomposed by carbonic acid but not by water*. The existence of such unsaturated functions was established even in soils containing as high as 6.6 per cent $CaCO_3$. From these experiments

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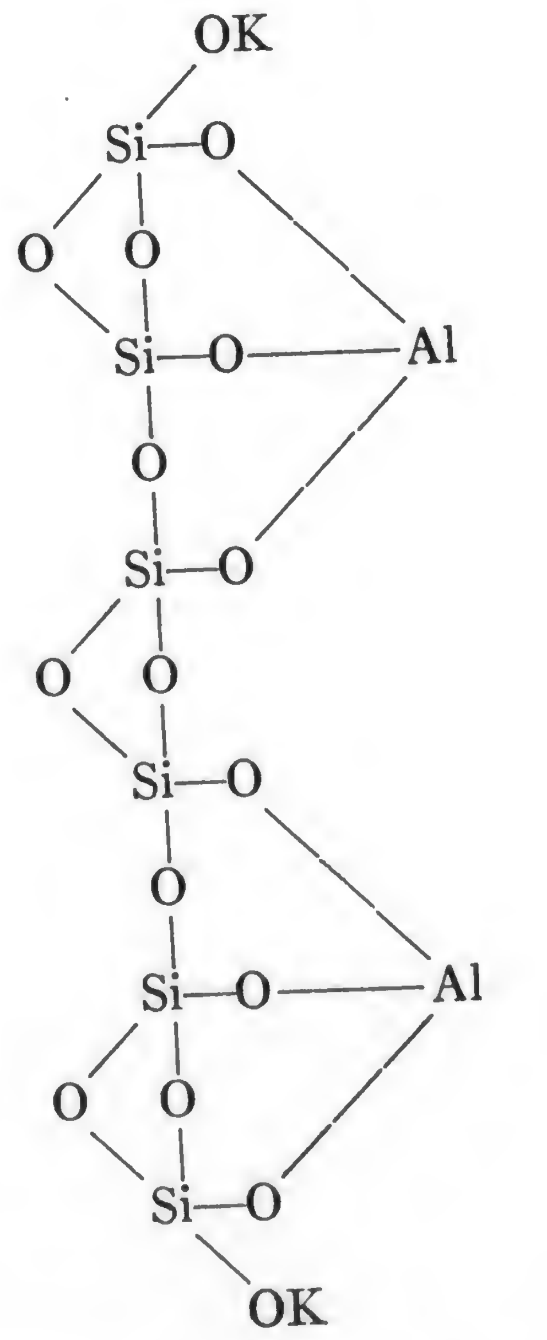
Clarens (5, 6, 9, 11, 12, 13) developed a method for the rapid characterization of a soil which the writer made use of on being confronted with difficulties in the interpretation of some of the results obtained in investigations on the condition of the phosphorus and also of the potassium in apple tree cylinder experiments (28, 31) by methods of continuous extraction with different dilute acids (30, 31).

The concept developed is essentially one that is at present generally accepted; namely, that the dominant exchange complex in soils of mineral origin consists of a series of alumino-silicic acids containing hydrogen ions of different activities (reaction values) (1, 3, 15, 24).

The constitution of the feldspars, kaolins, and clays

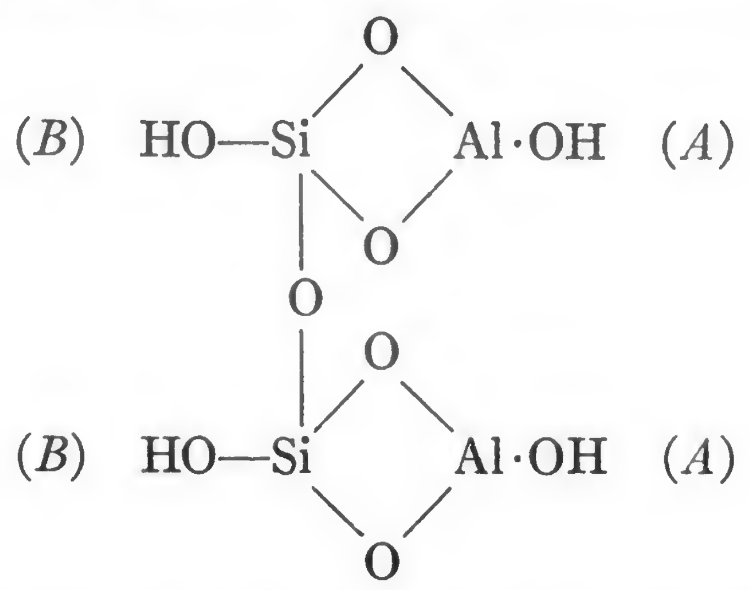
The constitutional formulas of the feldspars, kaolins, and clays represented below are not given as final but as indicating the convergence of our present knowledge. They serve as a means to the interpretation of the results of our experiments.

Basing our concepts of the constitution of the silicates of aluminum given by Clarke (14), we may deduce the structural formula of an unmixed orthoclase feldspar ($K_2Al_2Si_6O_{16}$)



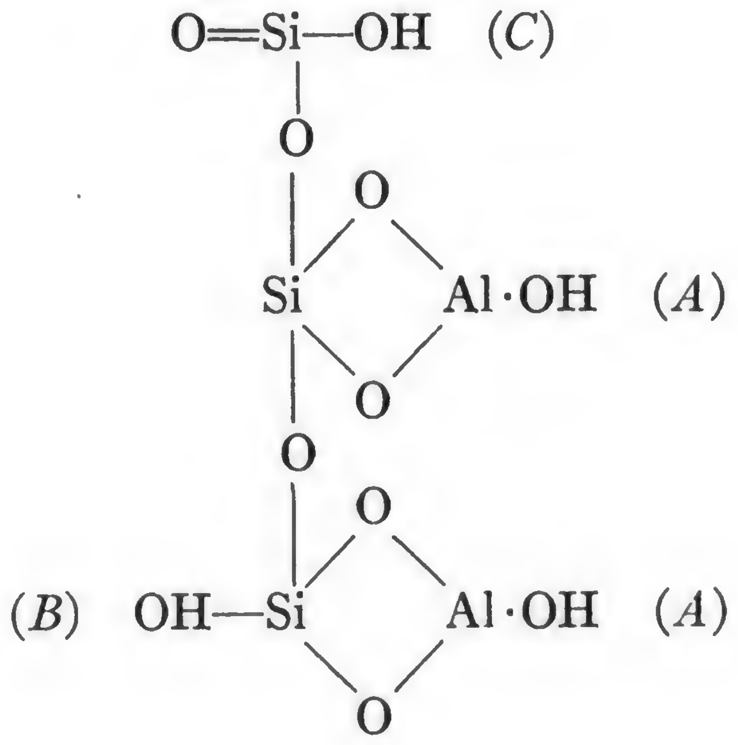
from one of the ortho-silicic acids (H_4SiO_4), by the elimination of 8 molecules of water from 6 molecules of H_4SiO_4 and the substitution of 6 hydrogen by 2

aluminum atoms and 2 hydrogen by 2 potassium atoms; and the constitution of a pure kaolin ($H_4Al_2Si_2O_9$)

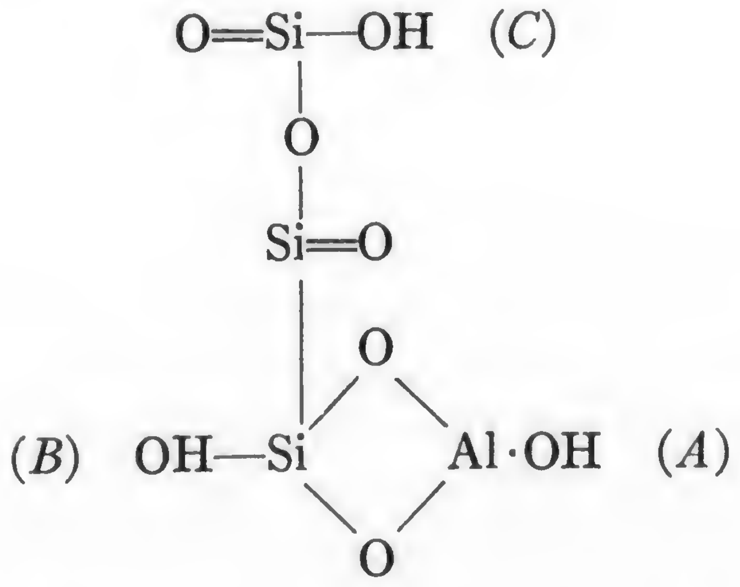


from orthoclase feldspar by the elimination of 4 molecules of SiO_2 and 2 of K.

In accordance with current concepts of the constitution of clay; namely, that clays are compounds of a series of alumino-silicic acids and feldspars, we may then deduce the structural formula for clay as typified by the following constitutional formulas:

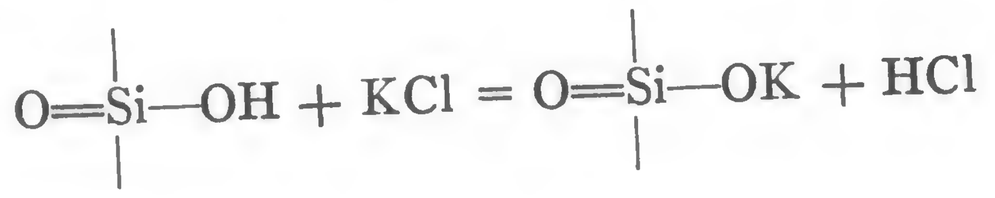


or (9)



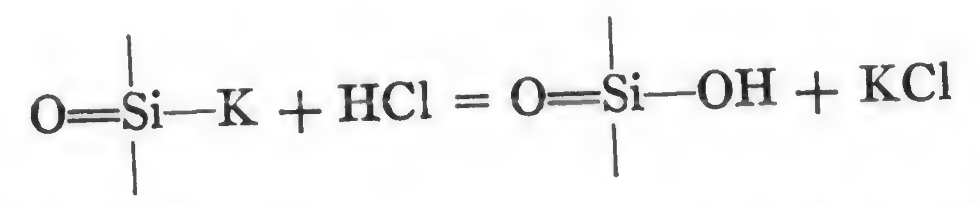
The three types of hydroxyl groups of clays

It is to be noted that the hydroxyl groups marked (*C*) in the formula for clay are not present in kaolin. Experimental evidence in support of this will be given later. Since these OH groups denoted by (*C*) are attached directly to Si atoms not directly linked to the Al atoms, they will possess distinct acidic properties—functioning as hydrogen silicate—and can be replaced partially or wholly by different metals, such as K, Ca, Mg.



$$O{=}\mathrm{Si}{-}OH + KCl = O{=}\mathrm{Si}{-}OK + HCl$$

and, conversely,

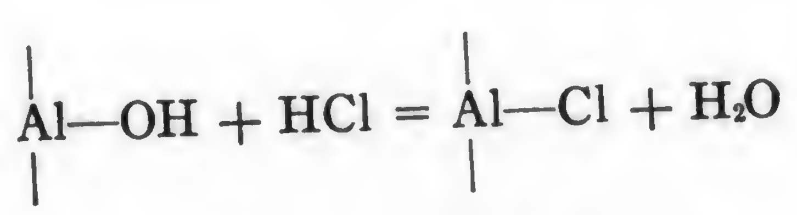


$$O{=}\mathrm{Si}{-}K + HCl = O{=}\mathrm{Si}{-}OH + KCl$$

Some of these hydroxyl groups marked (*C*) are free, and a portion of them may be replaced by bases. The free hydrogens can be determined by the quantity of $Ba(OH)_2$ *neutralized* in the formation of barium salts; and the hydrogens replaced by metals, by the quantity of HCl required to effect this substitution.

The hydroxyl groups marked (*B*) are linked to the Si atoms connected to Al atoms by an oxygen linkage. The hydrogens of these groups marked (*B*), therefore, will possess less pronounced acidic properties than those marked (*C*). With baryta the neutralization of the hydrogens of the OH groups marked (*B*) is only partial, since (*vide infra*) the barium salts of these acidic functions are hydrolyzed by water.

The hydroxyl groups denoted by (*A*) are linked directly to Al, an electro-positive element with basic properties. The salts obtained by the substitution of acid radicals in these OH groups undergo partial hydrolysis. The total replacement of these hydroxyl groups marked (*A*) can be obtained only when an excess of acid is used. When treated with an acid the amount of the acid radical fixed serves to evaluate the number of these hydroxyl groups substituted (*vide infra*)



$$\mathrm{Al}{-}OH + HCl = \mathrm{Al}{-}Cl + H_2O$$

MATERIALS AND METHODS

The original soil and also soils from two of the metal cylinders were used in these experiments. The soil designated as the "original" soil is the soil in its initial state before being introduced into the cylinders. It is a virgin soil of the Hagerstown series and has been fully described in an earlier publication (27): The soils designated "NPK" and "check" are representative of the samples of soils taken at the end of the experiment in the manner already described (29) from the surface 0–7 inches of the NPK treated and untreated cylinders under cultivation.

To 1-gm. portions of soil (sieved to pass a 100-mesh screen) in two series of tubes (from eight to ten tubes in each series) were added, to the one series, increasing quantities of 0.1 *N* HCl and, to the other, increasing amounts of 0.1 *N* $Ba(OH)_2$ in the following manner: To one tube 19 cc. of water and 1 cc. 0.1 *N* HCl (or 0.1 *N* $Ba(OH)_2$), to a second 18 cc. of water and 2 cc. of 0.1 *N* HCl (or 0.1 *N* $Ba(OH)_2$), and so on for eight to ten tubes. The volume of the solution in each tube is accordingly 20 cc. Freshly boiled distilled water was used. After leaving the soil in contact with the reagent for 18 hours, the tubes were continuously shaken for 6 hours. On settling, the solutions were filtered with suction with proper precautions in the case of $Ba(OH)_2$, and the number of cubic centimeters of 0.1 *N* HCl remaining in an aliquot (10 cc.) was titrated with methyl orange as indicator against standard alkali. The 0.1 *N* $Ba(OH)_2$

TABLE 1

Amounts of 0.1 N HCl and of 0.1 N $Ba(OH)_2$ remaining in solution after treatment of the "original" soil with increasing amounts of the acid and base

cc. 0.1 *N* HCl			cc. 0.1 *N* $Ba(OH)_2$		
Added	Remaining in solution	Fixed	Added	Remaining in solution	Fixed
1	0.20	0.80	1	0	1.00
2	1.05	0.95	2	0.30	1.65
3	1.90	1.10	3	1.10	1.90
4	2.75	1.25	4	1.90	2.10
5	3.60	1.40	5	2.70	2.30
6	4.55	1.45	6	3.65	2.35
7	5.55	1.45	7	4.65	2.35
8	6.55	1.45	8	5.65	2.35
9	7.40	1.60	9	6.50	2.50
10	8.30	1.70	10	7.35	2.65

TABLE 2

Amounts of 0.1 N HCl and of 0.1 N $Ba(OH)_2$ remaining in solution after treatment of the check soil with increasing amounts of the acid and base

cc. 0.1 *N* HCl			cc. 0.1 *N* $Ba(OH)_2$		
Added	Remaining in solution	Fixed	Added	Remaining in solution	Fixed
1	0.15	0.90	1	0	1.00
2	0.85	1.20	2	0.25	1.75
3	1.55	1.45	3	1.05	1.95
4	2.50	1.50	4	1.90	2.10
5	3.45	1.55	5	2.80	2.20
6	4.35	1.65	6	3.75	2.25
7	5.35	1.65	7	4.75	2.25
8	6.10	1.90	8	5.60	2.40
9	6.95	2.05	9	6.35	2.65
10	7.80	2.20	10	7.15	2.85

remaining in solution was titrated with phenolphthalein as indicator against standard acid.

For reasons the significance of which is pointed out later in this paper, the titration of the HCl remaining in solution is made first with methyl orange in the cold. The solution is then heated to boiling, and after the addition of a few drops of phenolphthalein the titration is continued to a permanent pink color.

TABLE 3

Amounts of 0.1 N HCl and of 0.1 N $Ba(OH)_2$ remaining in solution after treatment of the NPK soil with increasing amounts of the acid and base

cc. 0.1 *N* HCl			cc. 0.1 *N* $Ba(OH)_2$		
Added	Remaining in solution	Fixed	Added	Remaining in solution	Fixed
1	0.10	0.90	1	0	1.00
2	0.70	1.30	2	0.20	1.80
3	1.30	1.70	3	0.95	2.05
4	2.20	1.80	4	1.65	2.35
5	3.10	1.90	5	2.45	2.55
6	3.95	2.05	6	3.35	2.65
7	4.85	2.15	7	4.35	2.65
8	5.65	2.35	8	5.35	2.65
9	6.45	2.55	9	6.25	2.75
10	7.20	2.70	10	7.15	2.85

TABLE 4

Amounts of 0.1 N NaOH required to satisfy all bases remaining in solution, including Al and Fe, after treatment of the soils with increasing amounts of 0.1 N HCl

(Phenolphthalein titration of boiling solutions following the titration with methyl orange)

ORIGINAL		CHECK		NPK	
0.1 *N* HCl added	0.1 *N* NaOH required to neutralize all bases remaining in solution	0.1 *N* HCl added	0.1 *N* NaOH required to neutralize all bases remaining in solution	0.1 *N* HCl added	0.1 *N* NaOH required to neutralize all bases remaining in solution
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0.50	1	0.50	1	0.50
2	1.50	2	1.40	2	1.30
3	2.45	3	2.30	3	2.15
4	3.50	4	3.30	4	3.15
5	4.50	5	4.40	5	4.10
6	5.55	6	5.45	6	5.10
7	6.60	7	6.55	7	6.10
8	7.60	8	7.55	8	7.10
9	8.45	9	8.40	9	8.00
10	9.35	10	9.30	10	8.90

EXPERIMENTAL DATA

The results are given in tables 1 to 4 and are shown graphically in figures 1 to 3.

The "neutralization" portion of the graphs obtained for a particular soil consists of three segments; the equilibrium points making up each of the segments lie approximately on a straight line, the deviations being relatively small. The first segment is horizontal and, therefore, coincides with the abscissa; a second segment makes an angle of less than 45°; and a third segment has an angle the tangent of which is approximately unity, indicating saturation at the higher concentrations.

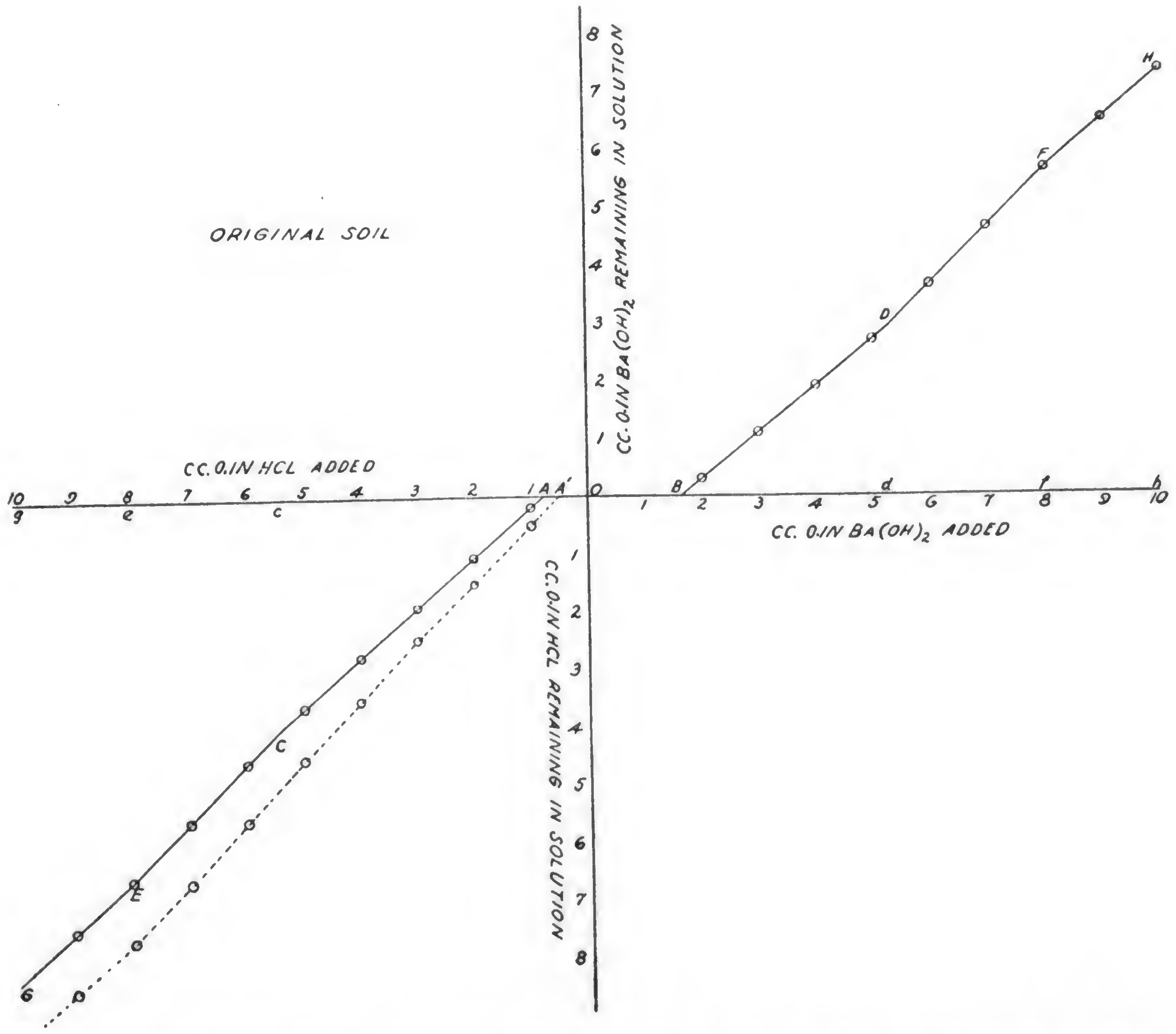


FIG. 1. THE "ORIGINAL" SOIL TREATED WITH SOLUTIONS OF 0.1 *N* HCl AND OF 0.1 *N* $Ba(OH)_2$

DISCUSSION OF RESULTS

The interpretation of the graphs

The results for the "original" soil, represented in figure 1, are described in detail to illustrate the method of interpretation.

The segment OB. In the "original" soil OB is equal to 1.62. This indicates that complete fixation of the baryta added has occurred up to a concentration of 1.62 cc. 0.1 *N* $Ba(OH)_2$ per gram of soil. The segment OB then evaluates those acidic functions the barium salts of which are not hydrolyzed by water free from CO_2 and corresponds to the unsaturated part of the hydroxyl groups denoted by (*C*) in the formula for clay previously given. Even in soils containing large amounts of $CaCO_3$, the segment OB will not be zero because of the presence of $Ca(HCO_3)_2$ in the soil solution. OB is only fully saturated by $Ba(OH)_2$ or, therefore, by $Ca(OH)_2$ when a slight excess of alkali is present.

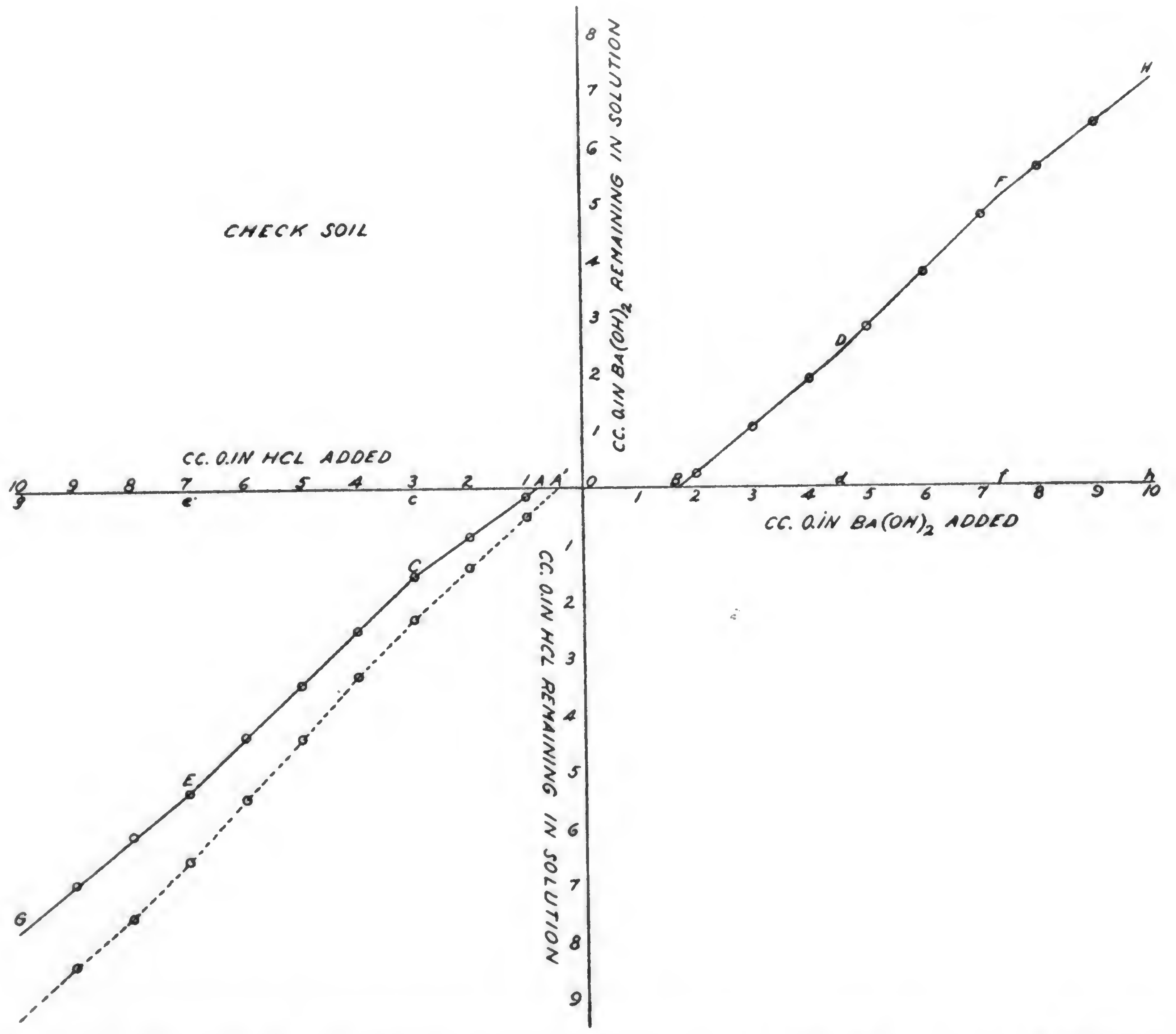


FIG. 2. THE "CHECK" SOIL TREATED WITH SOLUTIONS OF 0.1 *N* HCl AND OF 0.1 *N* $Ba(OH)_2$

The segment BD. After the replacement (neutralization) of the hydrogens represented by the segment OB, there still exist acidic functions the barium salts of which are partially hydrolyzed by water. This segment BD corresponds to those hydroxyl groups marked (*B*) and makes an angle—tan D_Bd = 0.82. This indicates that after a quantity of 0.1 *N* $Ba(OH)_2$ equal to OB (1.62 cc. per gram of soil) is added, the fixation of this base is only 18 per cent. The segment BD is evaluated thus: Bd − Dd = 3.40 − 2.70 = 0.70 cc. 0.1 *N* $Ba(OH)_2$ per gram of soil. The point d represents the intersection of the perpendicular from D to the abscissa. The acid functions represented by BD are of no practical importance, since the complete saturation of these groups would necessitate such an excess of base as would make the soil unfit for plants to thrive.

The segment DF. Commencing at D the third segment, DF, makes an angle of approximately 45° with the abscissa, indicating that the fixation of the base along this segment is nil, with formation of barium salts corresponding to other acidic functions having no affinity for $Ba(OH)_2$. Above a concentration of 8 cc. 0.1 *N* $Ba(OH)_2$ the curve again breaks and makes with the abscissa an angle the tangent of which is less than that made by DF. This segment FH has no bearing in these experiments. It is an indication of the instability of the clay complex, since above this concentration the baryta, instead of neutralizing, reacted with some of the components of the clay.

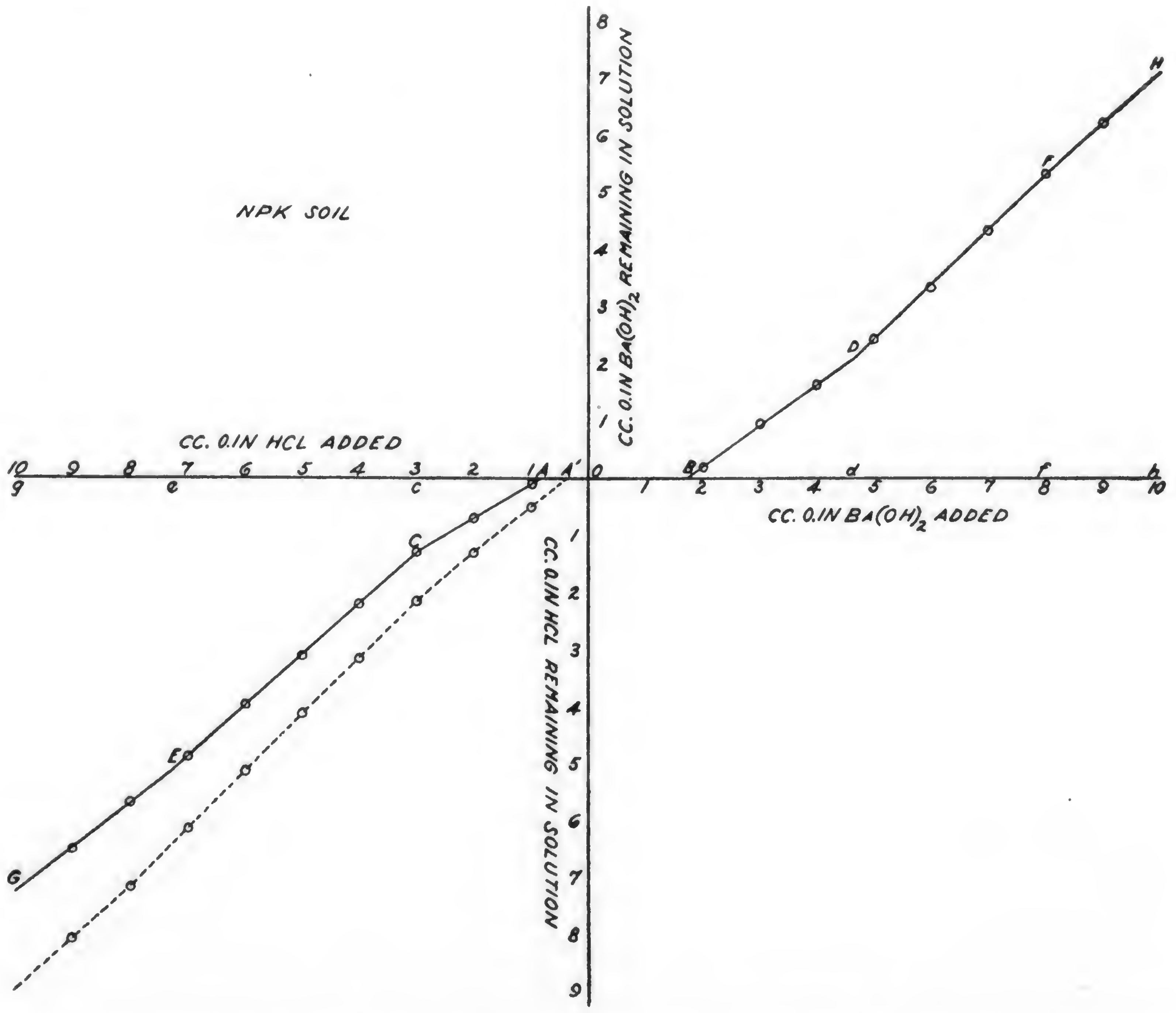


FIG. 3. THE "NPK" SOIL TREATED WITH SOLUTIONS OF 0.1 *N* HCl AND OF 0.1 *N* $Ba(OH)_2$

The segment OA. In the "original" soil OA is equal to 0.76 cc. In the absence of free bases, such as $Al(OH)_3$, $Fe(OH)_3$, $CaCO_3$, this segment would represent the number of cubic centimeters of 0.1 *N* HCl per gram of soil required to react with the exchangeable bases. OA then represents that fraction of the hydroxyl groups marked (*C*) the hydrogens of which have been replaced by metals.

If kaolin is substituted for soil in these experiments the points O, A, and B almost coincide (9). Even when digested with 1.7 *N* HCl for 10 hours in a boiling water bath, no decrease in the exchange capacity of either dickite or kaolin occurred (15). The segment AB, therefore, is relatively insignificant in kaolin, evidence that the hydroxyl groups marked (*C*) have, therefore, no equivalent in kaolin. The remainder of the graphs for kaolin do not differ from that for soils, i.e., there is a segment AC making an angle of less than 45° and a third segment the tangent of which is unity.

Although in the case of most of the soils examined by Clarens, free aluminum and iron hydroxides were absent, this is not the case with the Hagerstown soils reported here. The behavior of this Hagerstown clay loam to very low concentrations of HCl (0.000182 *M* to 0.00182 *M*) is intermediate to the behavior of kaolin and that of feldspar.

Whereas in kaolin the segment OA is insignificant, in feldspars the whole of this segment OA is represented by Al (9). An unmixed feldspar, examined by the writer, treated with increasing quantities of 0.1 *N* HCl titrated against 0.1 *N* alkali, using as indicators methyl orange followed by phenolphthalein, as described, subsequently, gave OA = 2.6 cc. 0.1 *N* NaOH per gram of feldspar and OA′ = 0.05. AA′, therefore, is equal to 2.55, showing that practically all of the exchangeable bases in this feldspar are represented by Al. The segment OB for feldspar was found to be 0.15 and, therefore, practically negligible.

It is evident then that the segment OA represents the exchangeable bases only if the soil contains no free bases or carbonates that may react with the acid employed. In the soils reported here the carbonates are relatively low (0.05 per cent), but appreciable quantities of "free" aluminum (and iron) are present. The fraction of the segment OA to be assigned to Al_2O_3 and Fe_2O_3 is represented in the figures by AA′ and can be obtained by titration with 0.1 *N* NaOH first at room temperature with methyl orange as indicator followed by titration of the solution raised to boiling temperature using phenolphthalein as indicator. A solution of $AlCl_3$ (or $FeCl_6$) neutral to methyl orange is acid to phenolphthalein. In titrating with 0.1 *N* NaOH starting from the point of neutralization with methyl orange, the phenolphthalein turns color only when all the aluminum and iron are precipitated as hydroxides. The difference between the two titration values with these indicators gives approximately, therefore, the quantity of $Al(OH_3)$ (and $Fe(OH)_3$) in solution in the form of $AlCl_3$ (or $FeCl_3$). The broken lines (fig. 1–3) and table 4 give the results with phenolphthalein as indicator.

In the "original" soil AA′ = 0.26 cc. gives the Al (and Fe) replaced per gram of soil. The exchangeable bases are represented by OA′ = 0.50 cc. 0.1 *N* HCl per gram of soil. In order to determine OA in calcareous soils, it is necessary to ascertain the quantity of 0.1 *N* acid necessary to neutralize the calcium carbonate in 1 gm. of soil. This quantity is then subtracted from the value obtained for the segment OA.

The segment AC. In the "original" soil the second segment AC makes an angle tan cAC = 0.84, indicating that the HCl has been fixed to the extent of 16 per cent. The value of AC is given by Ac − Cc = 0.7 cc. 0.1 *N* HCl per gram of soil, where c is the perpendicular from C on to the abscissa.

The acidic functions represented by the segment AC cannot be replaced by $Ba(OH)_2$. This has been repeatedly established (9) by treatment of soils which have previously been treated with increasing quantities of 0.1 *N* HCl in the manner already indicated, with increasing quantities of 0.1 *N* $Ba(OH)_2$ in amounts more than sufficient to effect complete substitution of all the hydrogen atoms. In all cases the hydrogen replaced by barium was always expressed by the value of the segment AB before the initial treatment with HCl. The segment AC then must correspond to the hydroxyl groups marked (*A*) in direct attachment with Al. Relative to the origin of the aluminum, Clarens states (10)[2]:

> This aluminum could have prevously existed in the free state or even have come from new silicates, which may either have remained attached to the rest of the molecule, so that spaces left by the aluminum might be filled by a metal of some sort, or have been carried off themselves in the form of SiO_2. The importance of these alternatives to the fate of feldspar that has become clay, or more accurately zeolite, is apparent.
>
> . . . It is evident that the feldspar which we have just been studying will not be transformed into zeolitic clay by the usual means (replacement of the alkaline and alkaline earth bases by various agents, particularly by H_2CO_3). It is the aluminum which will first be replaced, according to circumstances, by various bases which, in the resulting zeolitic clay, will constitute the replaceable bases; a small, usually very small, amount of the aluminum may perhaps remain and also act as a replaceable base. It is the potassium thus fixed that is called the "assimilable" potassium of the clay.

The segment CE. A third segment, CE, making an angle of 45° indicates that the acid functions represented are saturated and that no further fixation takes place.

On the addition of more acid the curve again breaks and makes an angle of less than 45°. Inasmuch as this indicates an attack on the clay nucleus, it is of no interest in these experiments.

The three segments of importance in application. The most important segments are OA, OB, and AC. OA evaluates, in the absence of free aluminum and ferric hydroxides and calcium carbonate, the acidic functions actually saturated by the so-called "replaceable bases"; OB evaluates free acidic functions the barium salts of which are not hydrolyzed by water free from CO_2 in the presence of a slight excess of base. The segment AC bears the same relation to acids, in particular to phosphoric acid, as the segment BD does to bases. This fact may be used to determine the amount of phosphoric acid

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[2] Translation from the original French.

necessary to satisfy the Al and Fe. It should be apparent that only four points are required to obtain all three segments.

Application to the determination of the amount of phosphoric acid required to satisfy the Al and Fe

In the "original" soil the angle which AC makes with the abscissa (tan = 0.84) indicates (assuming the fertilizer to be mixed thoroughly with the soil, which is never the case in practice) that if less than 0.84 cc. 0.1 *N* phosphoric acid per gram of soil be added the whole of the phosphoric acid would be fixed, resulting in a low concentration of phosphorus in the soil solution until this "threshold value" is exceeded.

Calculated as P_2O_5, the threshold value for the "original" soil is equal to

$$0.84 \times 0.002358 \times 585130 = 1159 \text{ gm. } P_2O_5$$

where 0.002358 is the number of grams of P_2O_5 in 1 cc. of 0.1 *N* acid and 585130 is the weight of the soil (in grams) in the 0 to 7-inch layer. Eleven hundred and fifty-nine grams of P_2O_5 is then required to saturate the segment represented by AC of the 0 to 7-inch layer in the "original" soil. Calculated to an acre basis of 2,000,000 pounds the value obtained is 3957 pounds P_2O_5, which is relatively enormous. In practice, the phosphate fertilizer is not intimately mixed with the whole of the first 7 inches. Under field conditions the diffusion of phosphate is very slow and frequently extends over a long period, thus allowing portions of the soil to be in contact with high concentrations of phosphate, and for this fraction the limiting value above is passed.

Application to determine lime requirement of soils

Many authorities are of the belief that there has been no satisfactory laboratory method developed thus far that can with certainty be substituted for the field-trial method for the determination of the lime requirements of soils. There is, however, a sound theoretical basis for the determination of the lime requirement by the present method.

It has frequently been assumed that when $CaCO_3$ is added to an acid soil the chief chemical reaction is with the so-called "replaceable" hydrogen ions of the exchange complex, i.e., the hydrogen ions replaceable by the base of a neutral salt. From the viewpoint presented it would not be logical to expect any definite relationship between the replaceable hydrogens and the degree of unsaturation of the exchange complex. Consequently, the determination of the replaceable hydrogen would not be a measure of the lime requirements of soils. The neutralization of replaceable H is only one of the factors involved in liming. Nor would it be in accordance with the concepts presented, to use as a basis for the lime requirement a quantity of $CaCO_3$ corresponding to the segment OB, for—because of the presence of $Ca(HCO_3)_2$ in the soil solution (see discussion of the segment OB)—this segment could never become nil even in soils in which large amounts of $CaCO_3$ has persisted for many years. The complete saturation of the segment OB can take place only in the presence of a slight excess of the hydroxide. It follows that the amount of $CaCO_3$ necessary for neutralization cannot be determined by shaking the soil with an excess of precipitated $CaCO_3$ and then determining the decrease in the segment OB. The saturation of the soil is very slow as a result of the low solubility of $CaCO_3$ even in the presence of small quantities of CO_2.

Clarens (8) has pointed out that if a soluble salt of calcium such as $CaSO_4$ or $CaCl_2$ be added also with the $CaCO_3$, the segment OB is markedly reduced and the reduction is independent within certain limits of the concentration (0.1 to 0.05 *N*) of the solution used. The reduction in the amount of OB then gives the quantity of $CaCO_3$ necessary for the effective saturation of the soil considered.

The experiments are conducted in the same manner as previously described. Five 1-gm. portions of soil are shaken up with 0.1 gm. of precipitated $CaCO_3$ and 20 cc. of a saturated solution of $CaSO_4$. The solutions are filtered with suction, and the soil is transferred to test tubes. To the first are added 19 cc. of water and 1 cc. 0.1 *N* $Ba(OH)_2$, to the second 18 cc. water and 2 cc. 0.1 *N* $Ba(OH)_2$, etc. The tubes are allowed to stand 18 hours, then shaken continuously for 6 hours, and then titrated in the usual manner against 0.1 *N* HCl and the new segment OB is determined. The "original" soil treated in this way gave OB = 1.10 as against 1.62 before treatment. Therefore, the saturation of the segment due to $CaCO_3 + CaSO_4$ is $1.62 - 1.10 = 0.52$ cc.

If the $CaCO_3$ is omitted and 20 cc. of a saturated solution of $CaSO_4$ only is used, the new segment OB is slightly greater than in the presence of $CaCO_3$ (0.59 as against 0.52). This shows that the $CaCO_3$ has caused a progression of 0 toward A of only 0.07. The conclusion must, therefore, be drawn that the saturation of the soil was due almost completely to the soluble salt of Ca, even when $CaCO_3$ is also present with the soluble salt (8).

This would explain why some investigators have found that when either $Ca(OH)_2$ or $CaCO_3$ is incorporated in one layer of soil (32) or applied as a top-dressing (16) the neutralizing effect is almost nil on the adjacent layers.

Application to determine the existence of different states of availability of the replaceable potassium

Of the many methods which have been proposed to determine the availability of soil potassium the determination of the replaceable form is the most rational and is the one generally favored. But it has already been shown (19) that broad generalizations based on the method are not possible. An additional limitation is imposed on the concept of the identification of the exchangeable potassium with the available potassium if the exchangeable potassium can possess different degrees of availability according to its position in the clay nucleus (7).

Reasoning *a priori* would lead one to expect that an exchangeable base such as potassium would not occupy the same position in the crystal lattice of the clay nucleus; and within the past few years experimental evidence has been brought to support this premise (4, 22, 23). It would follow, therefore, that the replaceable potassium should exist in different degrees of availability to the plant. If this is the case then the processes of mobilization in which the soil potassium is rendered available would be discontinuous and would be reflected by broken solubility curves.

The "original" surface soil has been investigated from this point of view in the following manner: 200-gm. portions of the "original" air-dry soil contained in 1000-cc. shaking flasks were treated with increasing amounts of 0.1 N HCl. To the first flask were added 975 cc. of distilled water and 25 cc. 0.1 N HCl; to the second, 950 cc. water and 50 cc. 0.1 N HCl; to the third, 925 cc. of water and 75 cc. 0.1 N HCl; and to the fourth, 900 cc. of water and 100 cc. 0.1 N HCl. The soils were then left in contact for 18 hours and then shaken continuously for 6 hours. Potassium was determined in an aliquot.

As shown in table 4, the replaceable bases of this "original" soil are equivalent to 0.50 cc. 0.1 N HCl per gram of soil. The amounts of HCl added to the flasks in this experiment accordingly were equivalent to one-fourth, one-half, three-fourths, and the whole of the replaceable bases, respectively. The results are given in table 5.

TABLE 5

Quantities of potassium liberated from the original soil by treatment with dilute solutions of HCl sufficient to satisfy 25, 50, 75, and 100 per cent, respectively, of the replaceable bases

0.1 N HCl ADDED	AMOUNT K_2O DISSOLVED	0.1 N HCl EQUIVALENT
cc.	*p.p.m.**	*cc.*
25	40	1.69
50	42	1.79
75	95	2.00
100	87	1.89

* Dry soil.

These results indicate that, within the range of concentration of HCl used, the solubility curve for the replaceable potassium is a discontinuous one.

Application to determine the changes produced by cultivation and fertilizer dressings

Although the aforementioned problems comprise the principal uses to which the method is adapted, it is of interest to ascertain what changes the method reveals in the properties of the initial condition of the soil at the end of the experimental period of 7 years. After introduction into the cylinders, both check and NPK soils were green manured each year with rye. The check soil received no mineral salts. The NPK soil received, during the whole experimental period of 7 years, total C. P. salt additions as follows: 465.5 gm. N, 1052.8 gm. P_2O_5, 1898 gm. K_2O—equivalent respectively to 1591 pounds N, 3598 pounds P_2O_5, 1898 pounds K_2O per acre 7 inches of 2,000,000 pounds. The precipitation during the whole experimental period was 260 inches, of which 96 inches fell during the experimental period in which the soil received mineral salts. All three soils are low in humus material. In spite of the green manuring, the humus material in the check and NPK soils was still relatively low, viz., <1.5 per cent.

The summarized results of the analytical data for the "original" soil and also for the check and NPK soils are given in table 6.

The divergences in the results of the check soil from the "original" soil are an indication of the joint effects of the growth of the trees and of the humus material from the green manures together with the climatic factors. The degree of unsaturation as represented by the segment OB has increased the equivalent of 0.06 cc. 0.1 N $Ba(OH)_2$ per gram of soil. The replaceable bases in the check soil have been reduced by the equivalent of 0.05 cc. 0.1 N HCl per gram of soil, whereas the "free" aluminum and iron have been increased by 0.09 cc. 0.1 N HCl per gram of soil. A decrease in the tangent of the angle CAc from 0.84 to 0.72 has occurred, which indicates an increment from a saturation of 16 per cent to a saturation of 28 per cent in the hydroxyl groups marked (A). The most rational explanation would appear to be that this increase is the result of the "deactivating" properties of humic acids (30).

TABLE 6

Comparative data for the original soil, the check, and NPK cylinders

SOIL	SEGMENT OA	SEGMENT OA′	SEGMENT AA′	SEGMENT OB	TAN $<$ CAc	TAN $<$ dBD
Original	0.76	0.50	0.26	1.62	0.84	0.82
Check	0.80	0.45	0.35	1.68	0.72	0.79
NPK	0.85	0.43	0.42	1.73	0.60	0.73

OA = Acid functions saturated by bases including free Fe and Al expressed in terms of cubic centimeters of 0.1 N HCl per gram of soil.

OA′ = Acid functions saturated by the so-called "replaceable bases" expressed in terms of cubic centimeters of 0.1 N HCl per gram of soil.

AA′ or OA − OA′ = "Free" $Al(OH)_3$ and $Fe(OH)_3$ expressed in terms of cubic centimeters of 0.1 N HCl per gram of soil.

OB = Unsaturated acidic functions the Ba salts of which are not hydrolyzed by water free from CO_2 in the presence of a slight excess of base. Expressed in terms of cubic centimeters of 0.1 N $Ba(OH)_2$ per gram of soil.

The values for the NPK soil differ more widely from the corresponding values for the "original" soil than do those of the check soil. Particularly to be noted is the *increase* of the segment OB equivalent to 0.11 cc. 0.1 N $Ba(OH)_2$ per gram of soil; the *decrease* equivalent to 0.07 cc. 0.1 N HCl per gram of soil in the replaceable bases; and the relatively large increase equivalent to 0.16 cc. 0.1 N HCl per gram of soil in the "free" aluminum and iron hydroxides. The decrease in the tangent of the angle CAc is equivalent to 0.24, indicating an increase of 24 per cent in the saturation of the hydroxyl groups marked (A) and representing the net effect of the deactivating properties of humic acids and the phosphate additions. In laboratory studies, Clarens (5) has reached the conclusion that salts of K, Na, NH_4, and Ca do not sensibly modify the unsaturated acid functions. The present experiment indicates that the resultant effect of the $NaNO_3$, K_2SO_4, and $CaH_4(PO_4)_2$ added as fertilizers upon the segments OA, AA′, and OB is similar in magnitude and direction to that produced by the green manuring alone. Moreover, it is a noteworthy fact that the salt additions have not caused any increase in the segment OA′ but, on the contrary, have diminished it slightly. It is the segment AA′ that has increased evidently at the expense of the so-called "replaceable bases."

SUMMARY

By the treatment of a soil with increasing amounts of solutions of hydrochloric acid and also of barium hydroxide in low concentrations, differences in specific properties of the three types of hydroxyl groups of clays may be used as a means for the rapid characterization of a soil with respect to the degree of unsaturation, the total replaceable bases, the "free" aluminum and iron hydroxides, the phosphoric acid required to satisfy the Al and Fe, the lime requirement, the availability of the different states of the replaceable potassium, and the changes produced by cultivation and fertilizer treatment.

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THE DISTRIBUTION AND CONDITION OF THE POTASSIUM IN A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN CYLINDERS

BY

WALTER THOMAS
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THE DISTRIBUTION AND CONDITION OF THE POTASSIUM IN A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN CYLINDERS[1]

By WALTER THOMAS

Professor of phytochemistry, Pennsylvania Agricultural Experiment Station

INTRODUCTION

In an earlier paper (*19*)[2] the writer reported the utilization and recovery of nitrogen, phosphorus, and potassium by apple trees grown in metal cylinders for a period of 6½ years. These trees received (each spring) during the last 3 years of growth different combinations of the pure salts sodium nitrate, monocalcium phosphate, and potassium sulphate. The ratio in which nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) were absorbed from the added salts by the trees which gave optimum growth and reproduction, viz, those growing in the NPK- and NP-treated cylinders, was 3.0:0.3:1.5, whereas the ratio in which these same fertilizer entities were applied was 3:8:4. This latter ratio is based on current orchard practice. Because of the great divergence in these ratios studies were made to determine the distribution and condition of the nitrogen, phosphorus, and potassium in the soil at the end of the experiment. The results as they relate to nitrogen and phosphorus have already been reported (*20, 21*). The corresponding studies were made to determine the distribution and condition of the nitrogen, phosphorus, and potassium in the soil at the end of the experiment. The results as they relate to nitrogen and phosphorus have already been reported (*20, 21*). The corresponding data for potassium are given in the present paper.

Although both phosphorus and potassium are "fixed" by soils, the solubility relations of these elements are markedly different. The phosphorus concentration of the soil solution of a particular soil varies over a relatively small range, whereas that of potassium fluctuates over a wide range even when the soil neither receives applications of potassium salts nor suffers loss by leaching or removal by the plant.

In this paper all results have been calculated to and reported as the oxide K_2O, commonly designated potash. Occasionally, in the text, reference is made to the element potassium where the use of the ionic form appears to be the more suitable. The reader, however, should keep in mind that all values given in the tables are expressed as the oxide K_2O.

METHODS

THE FIELD PLAN

Since a detailed plan of this experiment has already been presented (*1, 20, 21*), only an outline of the procedure will be given here. A virgin soil (*18*) of Trenton formation, formed by the weathering of

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[1] Received for publication Feb. 6, 1936; issued November 1936. Paper no. 736 of the Journal series of the Pennsylvania Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 545.

limestone, was used. This soil is designated throughout as the "original" soil, and represents the condition of the soil before the experiment began. The method of excavation, the preparation and mixing of each horizon, and the filling of the cylinders have been described in detail (*18*, *20*) and also the method of sampling the soil before and after the experiment (*21*).

The sources of the potassium in this Hagerstown soil are the mixed feldspars, micas, and tourmalines. The silt fraction contains a preponderance of microcline with some quartz; whereas the fine sand (0.1 to 0.5 mm) consists of 50 percent of mixed feldspars and 35 percent of quartz. Orthoclase is present in very small amounts and is highly kaolinized. No fresh unaltered samples of orthoclase exist in this soil (*18*).

For the first 2 years after the trees were planted the culture system was uniform in all cylinders. It consisted of green manuring with buckwheat and rye principally. Commencing with the third year, the system was differentiated as follows: Half of the cylinders were kept under a system of clean cultivation. These cylinders are designated "cylinders under cultivation." Half the cylinders were seeded with a mixture of bluegrass and timothy. These cylinders are designated "cylinders under sod." The schedule of applications of potassium sulphate is given in table 1.

TABLE 1.—*Schedule of potassium sulphate applications with potash equivalent*

Date applied	K_2SO_4	K_2O equivalent
	Grams	*Grams*
Apr. 18, 1925	293	158.45
May 3, 1926	147	79.49
June 7, 1926	147	79.49
May 5, 1927	293	158.45
May 18. 1927	147	79.49
Total	1,027	555.37

The total amount of potash added in the fertilizer during the whole period of the experiment was, as indicated above, 555.4 g. In addition, 25 g of potash was added in the form of cover crops of all cylinders up to the period of differentiation referred to in the last paragraph. An additional 15 g of potash was added after this period, but to the cylinders under cultivation only. The purpose in making these relatively large applications of fertilizers was to saturate the fixing capacity of the 0- to 7-inch layer of the original virgin soil with respect to phosphoric acid. The amounts of nitrogen and potash applied were porportionally increased so as to maintain the ratio 3:8:4.

LABORATORY EXPERIMENTS

The method of approach in determining the condition of the potassium was to find, in addition to the total potassium, the amounts removed by continuous leaching with three solvents, namely, (1) freshly boiled distilled water, (2) M/2 ammonium acetate, and (3) 0.2 N nitric acid. A few experiments were also carried out with 1-percent citric acid, but these experiments are not recorded inasmuch as no additional information was obtained by this reagent.

The total potash (K_2O) was determined in the three horizons of all soils by the J. Lawrence Smith method (*22*), a 0.5 g charge of finely pulverized soil being used. The detailed results for total potash are not recorded for reasons which will be apparent later (p. 538).

The continuous leaching experiments were conducted on 10 g charges of soil in the same manner and with the same apparatus that was described and illustrated in a preceding paper (*21*). The temperature of the laboratory during the experiments varied between 75° and 85° F. Preliminary experiments showed that the increase in the amounts of potash extracted per 2° rise in temperature was equivalent to 0.9 percent of that extracted at 75°. This is relatively a negligible increase. The rate of percolation was approximately 1 drop in 90 seconds.

The potash content of the leachates was determined by three methods. When the amounts were sufficiently large for gravimetric procedure the potassium was precipitated and weighed as the chloroplatinate (*2*) after the removal of silica, iron, alumina, calcium, and barium. When, however, the amount of potassium extracted reached values below 1 mg in 200 cc of the extract, recourse was had to the sodium cobaltinitrite method as devised by Drushel (*7*, *8*) and modified by Morris (*14*). Further improvements have been introduced by Hibbard (*11*).

The determinations of such extremely low values of magnitude as were given in all the extracts with distilled water and with ammonium acetate after the first leachate were carried out by the colorimetric method of Schreiner and Failyer (*17*, *pp. 31–33*).

Difficulty has been experienced by some investigators with the platinic chloride method, especially on the extracts from ammonium salts, which are usually very high in calcium. When this method is used it is necessary for the analyst to assure himself of the complete removal of all traces of calcium before proceeding to the precipitation of the mixed chloroplatinates. This may be accomplished by treating the sirupy mixed chloroplatinates with 15 to 20 cc of a solution of acidified alcohol. The acidified alcohol is prepared by passing washed hydrochloric acid gas into commercial 95-percent alcohol until the solution is two to three times normal in strength. The usual procedure is then followed (*2*).

EXPERIMENTAL DATA

The total potash (K_2O) content of the three horizons of the original, check, NPK, NK, and PK soils was found to range between certain maximal and minimal values shown in the following tabulation:

Horizon	*Range of K_2O, percent*
Surface soils (0 to 7 inches)	3.919 to 4.007
Subsurface soils (7 to 21 inches)	4.197 to 4.205
Subsoils (21 to 53 inches)	4.405 to 4.410

The probable error of analyses may be gaged from 10 determinations made on the original surface soil before the experiment began. The results were 3.925±0.005.

The results of the percolation experiments with water, M/2 ammonium acetate, and 0.2 N nitric acid are given in table 2.

TABLE 2.—*Potash (K_2O) in successive leachings with distilled water, M/2 ammonium acetate, and 0.2 N nitric acid, expressed in parts per million of dry soil and in grams*

DISTILLED WATER

Description of soil	Potash in successive leaching indicated										Total in first 3 leachings	Absolute amount in first 3 leachings	Total in all leachings	Absolute amount in all leachings
	1	2	3	4	5	6	7	8	9	10				
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Grams*	*P. p. m.*	*Grams*
Soil before trees were planted:														
0–7 inches	41.4	17.2	10.4	9.8	10.0	7.2	6.0	5.2	5.0	5.0	69.0	40.4	117.2	68.6
7–21 inches	27.9	12.0	9.8	10.0	8.5	6.4	6.4	6.0	5.5	5.5	49.7	58.6	98.0	115.6
21–53 inches	26.2	11.4	10.2	9.3	9.1	7.5	6.2	6.0	4.0	4.0	47.8	128.6	93.9	252.6
Check (sod):														
0–7 inches	44.7	15.2	12.7	10.1	9.4	10.0	9.9	9.7	9.8	9.8	72.6	42.5	141.3	82.7
7–21 inches	33.4	11.4	9.7	10.0	9.8	9.2	9.4	10.0	8.9	9.0	54.5	64.3	120.8	142.5
21–53 inches	26.9	10.7	10.3	9.0	9.1	8.0	9.4	9.7	9.0	9.0	47.9	128.8	111.1	298.8
Check (cultivation):														
0–7 inches	51.7	18.9	12.4	10.2	11.0	9.2	9.2	9.5	9.5	9.5	83.0	48.6	151.1	88.4
7–21 inches	37.5	14.2	10.7	9.0	9.5	9.5	8.0	9.0	9.0	9.0	62.4	73.6	125.4	147.9
21–53 inches	32.1	12.7	9.2	8.5	9.4	9.0	9.5	9.0	8.5	8.5	54.0	145.2	116.4	313.1
NPK (sod):														
0–7 inches	190.0	42.6	32.6	21.3	15.7	10.9	9.8	10.2	10.4	9.8	265.2	155.2	353.3	206.7
7–21 inches	77.2	27.4	15.6	10.8	11.1	9.2	9.2	9.0	9.5	9.0	120.2	141.7	188.0	221.7
21–53 inches	27.9	14.9	10.7	9.5	9.5	10.0	9.0	9.0	9.5	9.0	53.5	143.9	119.0	320.1
NPK (cultivation):														
0–7 inches	201.0	62.4	24.6	12.4	10.9	11.0	9.0	8.4	9.0	9.0	288.0	168.5	357.7	209.3
7–21 inches	82.6	29.4	14.2	10.0	9.0	9.0	8.5	8.5	8.5	8.5	126.2	148.8	188.2	221.9
21–53 inches	29.0	13.6	10.4	9.1	10.0	9.5	9.5	9.0	9.0	9.0	53.0	142.5	118.1	317.7
PK (sod):														
0–7 inches	177.4	35.4	[1] 27.9	--------	--------	--------	--------	--------	--------	--------	240.7	140.8	--------	--------
7–21 inches	80.4	25.2	[1] 12.9	--------	--------	--------	--------	--------	--------	--------	118.5	139.8	--------	--------
21–53 inches	25.7	16.2	[1] 9.4	--------	--------	--------	--------	--------	--------	--------	51.3	138.0	--------	--------
PK (cultivation):														
0–7 inches	188.2	33.7	[1] 25.4	--------	--------	--------	--------	--------	--------	--------	247.3	144.7	--------	--------
7–21 inches	90.4	25.6	[1] 12.2	--------	--------	--------	--------	--------	--------	--------	128.2	151.2	--------	--------
21–53 inches	28.2	12.9	[1] 7.2	--------	--------	--------	--------	--------	--------	--------	48.3	129.9	--------	--------
M/2 AMMONIUM ACETATE														
Soil before trees were planted:														
0–7 inches	73.6	17.4	15.5	10.2	8.0	--------	--------	--------	--------	--------	106.5	62.3	124.7	73.0
7–21 inches	101.3	35.3	17.9	12.4	8.5	--------	--------	--------	--------	--------	154.5	182.2	175.4	206.8
21–53 inches	160.8	51.0	22.4	11.2	9.0	--------	--------	--------	--------	--------	234.2	629.9	254.4	684.3
Check (sod):														
0–7 inches	106.1	34.8	17.4	12.1	9.5	--------	--------	--------	--------	--------	158.3	92.6	179.9	105.3
7–21 inches	106.0	50.3	29.6	13.4	9.0	--------	--------	--------	--------	--------	185.9	219.2	208.3	245.6
21–53 inches	172.9	44.5	22.6	12.8	8.2	--------	--------	--------	--------	--------	240.0	645.5	261.0	702.0
Check (cultivation):														
0–7 inches	112.2	37.4	16.2	12.0	8.5	--------	--------	--------	--------	--------	165.8	97.0	186.3	109.0
7–21 inches	116.0	52.4	28.7	14.2	9.0	--------	--------	--------	--------	--------	197.1	232.4	220.3	259.8
21–53 inches	160.5	51.8	22.7	10.4	8.8	--------	--------	--------	--------	--------	235.0	632.1	254.2	683.7
NPK (sod):														
0–7 inches	329.4	51.4	24.3	12.2	9.4	--------	--------	--------	--------	--------	405.1	237.0	426.7	249.7
7–21 inches	204.8	51.2	24.9	11.8	9.0	--------	--------	--------	--------	--------	280.9	331.3	301.7	355.8
21–53 inches	167.9	48.4	23.2	12.4	8.0	--------	--------	--------	--------	--------	239.5	644.2	259.9	699.1
NPK (cultivation):														
0–7 inches	322.4	50.2	30.4	12.1	10.0	--------	--------	--------	--------	--------	403.0	235.8	425.1	248.7
7–21 inches	219.2	53.7	27.8	11.0	10.2	--------	--------	--------	--------	--------	300.7	354.6	321.9	379.6
21–53 inches	163.8	49.6	24.9	10.2	9.5	--------	--------	--------	--------	--------	238.3	641.0	258.0	694.0
PK (sod):														
0–7 inches	342.4	60.4	[1] 28.2	--------	--------	--------	--------	--------	--------	--------	431.0	252.2	--------	--------
7–21 inches	200.8	47.2	[1] 22.8	--------	--------	--------	--------	--------	--------	--------	270.8	319.4	--------	--------
21–53 inches	168.2	50.4	[1] 26.4	--------	--------	--------	--------	--------	--------	--------	245.0	659.0	--------	--------
PK (cultivation):														
0–7 inches	319.6	58.2	[1] 39.4	--------	--------	--------	--------	--------	--------	--------	417.2	244.1	--------	--------
7–21 inches	210.4	52.4	[1] 29.2	--------	--------	--------	--------	--------	--------	--------	292.0	344.4	--------	--------
21–53 inches	162.9	45.6	[1] 23.4	--------	--------	--------	--------	--------	--------	--------	231.9	623.8	--------	--------
0.2 N NITRIC ACID														
Soil before trees were planted:														
0–7 inches	196.3	104.7	72.4	53.9	37.4	29.6	25.4	23.2	22.4	20.6	373.4	218.5	585.9	342.8
7–21 inches	235.5	182.1	170.5	135.6	117.2	83.4	48.9	39.7	28.6	25.0	588.1	693.6	1,066.5	1,257.8
21–53 inches	347.8	213.1	187.9	168.6	149.2	92.1	70.6	49.4	37.4	30.6	748.8	2,014.1	1,346.7	3,622.3
Check (sod):														
0–7 inches	360.4	251.9	224.8	143.4	135.6	116.2	89.1	54.2	38.7	33.8	837.1	489.8	1,448.1	847.3
7–21 inches	392.6	203.4	156.9	143.3	120.6	112.4	91.1	42.6	39.7	35.4	752.9	887.9	1,338.0	1,577.9
21–53 inches	372.1	203.5	172.4	158.4	136.8	101.4	81.6	53.2	38.9	35.6	748.0	2,012.0	1,353.9	3,641.7
Check (cultivation):														
0–7 inches	347.3	262.6	219.8	144.6	130.8	119.7	88.6	52.4	39.6	38.4	829.7	485.5	1,443.8	844.8
7–21 inches	400.0	224.8	158.9	140.4	118.7	104.2	99.7	59.4	48.2	41.4	783.7	924.2	1,395.7	1,646.0
21–53 inches	370.4	200.6	158.4	150.2	144.5	102.4	81.4	71.8	52.6	40.9	729.4	1,961.9	1,373.2	3,693.6
NPK (sod):														
0–7 inches	830.0	244.2	235.9	135.0	96.9	88.4	77.5	61.0	42.1	38.4	1,310.1	766.6	1,849.4	1,082.1
7–21 inches	446.8	262.8	160.4	149.8	120.0	110.7	98.2	50.6	48.1	40.4	870.0	1,026.0	1,487.8	1,754.6
21–53 inches	382.2	208.4	178.4	172.8	137.8	112.4	91.4	63.8	58.9	42.6	769.0	2,068.4	1,448.7	3,896.7
NPK (cultivation):														
0–7 inches	817.4	239.4	[1] 220.6	--------	--------	--------	--------	--------	--------	--------	1,277.4	747.4	--------	--------
7–21 inches	459.2	260.4	[1] 179.4	--------	--------	--------	--------	--------	--------	--------	899.0	1,060.2	--------	--------
21–53 inches	370.6	208.0	[1] 190.4	--------	--------	--------	--------	--------	--------	--------	769.0	2,068.4	--------	--------
PK (sod):														
0–7 inches	848.8	147.2	135.6	116.2	116.2	100.6	93.0	77.2	60.4	40.1	1,131.6	662.1	1,735.3	1,015.4
7–21 inches	447.2	260.4	162.8	160.4	119.0	104.6	94.2	70.4	58.4	40.4	870.4	1,026.5	1,517.8	1,789.9
21–53 inches	383.8	208.4	178.6	166.4	138.2	109.4	92.8	70.4	42.8	31.4	770.8	2,073.3	1,422.2	3,825.4
PK (cultivation):														
0–7 inches	839.4	154.2	[1] 141.4	--------	--------	--------	--------	--------	--------	--------	1,135.0	664.1	--------	--------
7–21 inches	462.8	278.0	[1] 190.4	--------	--------	--------	--------	--------	--------	--------	931.2	1,098.2	--------	--------
21–53 inches	380.8	202.2	[1] 168.4	--------	--------	--------	--------	--------	--------	--------	751.4	2,110.7	--------	--------

[1] Percolation experiments were not carried further.

ANALYSIS AND DISCUSSION OF RESULTS

DOWNWARD MOVEMENT OF POTASSIUM

As Determined From Data for Total K_2O

The error involved in the determination of total potash, although relatively small, is absolutely large when multiplied by the weights of the soil in each horizon, viz, surface 1,290 pounds, subsurface 2,600 pounds, and subsoil 5,930 pounds. When the data for the total potash from each horizon were analyzed it was found that the error of analysis was too great to permit conclusions to be drawn with respect to the movement of potassium applied as fertilizer.

As Determined From Percolation Data

More accurate information should be obtained from extraction or percolation data. The amount of potassium which has moved from one horizon to another can be obtained by the difference between the amounts removed by the respective solvents from corresponding horizons of treated and check cylinders. The data so obtained are shown in table 3.

Table 3.—*Increase in the amounts of K_2O removed in 3 leachings by distilled water, M/2 ammonium acetate, and 0.2 N nitric acid from the NPK and PK cylinders above that of the corresponding horizons of the check cylinders*

Description of soil	Distilled water	M/2 ammonium acetate	0.2 N nitric acid
	Grams	*Grams*	*Grams*
NPK (sod):			
0-7 inches	113	144	277
7-21 inches	77	108	138
21-53 inches	6	−[1] 5	56
NPK (cultivation):			
0-7 inches	120	139	262
7-21 inches	75	127	136
21-53 inches	−[1] 1	9	53
PK (sod):			
0-7 inches	98	160	172
7-21 inches	76	96	139
21-53 inches	3	17	61
PK (cultivation):			
0-7 inches	98	147	180
7-21 inches	77	117	174
21-53 inches	−[1] 6	−[1] 8	60

[1] Decrease.

The progressive increases in these values (table 3) as one proceeds from the distilled water, M/2 ammonium acetate, and 0.2 N nitric acid extracts in the 7- to 21-inch and 21- to 53-inch horizons of the respective treatments indicate the condition with respect to solubility, in each solvent, of the applied potassium sulphate that has moved downward. The quantities of potassium that have so moved are expressed as a percentage of the residual applied potash in table 4. The residual amounts can be calculated from the known amounts of potash applied and that absorbed by the trees (*19*).

The subsurface data show that amounts ranging from 14.1 to 15.3 percent of the portion of the residual applied potash which has moved into the 7- to 21-inch layer are soluble in water; that amounts ranging from 17.9 to 26.4 percent are removed by M/2 ammonium acetate and 27.1 to 32.1 percent by 0.2 N nitric acid.

Table 4.—*Fraction of the applied potash found in the different leachates from the subsurface and subsoil horizons expressed as a percentage of the residual applied potash*

Horizon and treatment	Distilled H_2O	M/2 $NH_4.C_2H_3O_2$	0.2 N HNO_3
	Percent	*Percent*	*Percent*
Subsurface:			
NPK (sod)	15.1	21.0	27.1
NPK (cultivation)	15.3	26.4	28.3
PK (sod)	14.1	17.9	25.5
PK (cultivation)	14.2	21.6	32.1
Subsoil:			
NPK (sod)	1.2	----------	11.0
NPK (cultivation)	----------	1.9	11.0
PK (sod)	.5	3.2	11.4
PK (cultivation)	----------	----------	11.0

The subsoil data are more difficult to interpret. The percentages of the residual potash removed by distilled water and M/2 ammonium acetate probably lie within the limits of error of the experiment. The high results for the amounts removed by 0.2 N nitric acid therefore may not indicate a downward movement into this horizon, but may be a reflection of the increased solubility in this solvent of the native soil potash as the result of the effect of the far greater root systems in the treated cylinders.

CONDITION OF THE POTASSIUM

The Water-Soluble Potassium

Since distilled water will remove the salts that have not been adsorbed by the clay and humus complexes of the soil, leaching with this solvent in in effect similar to that of leaching with an electrolyte. Brönsted's investigations (*4*, *5*) indicate the character of solubility changes in the presence of electrolytes.

The concentration of potassium, which is relatively high in all of the first percolates with distilled water, rapidly falls in the second extracts, and the decrease is then more gradual as the electrolytes of the soil solution are removed. This gradual removal of electrolytes explains why the fraction of the so-called exchangeable potassium (p. — and table 5) removed by distilled water prior to the establishment of a constant concentration is so large.

Table 5.—*The quantities of so-called exchangeable potash removed in the first 3 leachings with distilled water*

Description of soil	Original soil	Check		NPK		PK	
		Sod	Cultivation	Sod	Cultivation	Sod	Cultivation
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Surface	64.8	45.9	50.1	65.5	71.5	55.8	59.3
Subsurface	32.2	29.3	31.7	42.8	42.0	43.8	43.9
Subsoil	20.4	19.9	23.0	22.3	22.2	20.9	20.8

The results show how impossible it is to determine the real quantities of water-soluble and exchangeable potassium. The water-soluble potassium must necessarily contain a certain amount of exchangeable potassium, because the leaching with water of these soils containing relatively large amounts (p. 534) of applied potassium sulphate is in fact equivalent to leaching with a dilute solution of a neutral salt—in this case potassium sulphate—containing no ion in common with the salt used to determine the replaceable potash, viz, ammonium acetate. This phenomenon is further discussed in the next two sections from the point of view of the more general aspect of soil problems. Moreover, the ammonium acetate leachates will necessarily contain the water-soluble potash. It is, therefore, obvious that the "replaceable" potash cannot be definitely and exactly determined.

The very large percentages of the so-called exchangeable potash removed by water in these experiments is consequently explicable. Thus, from 46 to 50 percent of the so-called exchangeable potassium (p. 541) is removed by distilled water in the first three extracts from the surface soil of the check cylinders and from 65 to 71 percent from the NPK cylinders. Even in the surface layer of the original soil 65 percent of the exchangeable potassium is removed by water in the first three extracts. In the lowest horizons the fraction of the exchangeable potassium removed in the first three extracts is reduced to approximately 20 percent.

In the check and all treated cylinders the concentration of potassium in the eighth and succeeding leachates with distilled water is reduced to approximately 10 parts per million of the dry weight of the soil. In the original soil the concentration tends toward a relatively constant level of about 5 parts per million as leaching is continued. These differences in the constant level reached by the original soil and in the soils in the cylinders at the end of the experiment are significant. The stage reached by reduction to the constant level is indicative of the existence of a potassium combination of such low solubility that the amount going into solution at each extraction is independent of the mass present in the soil. The influences to which both the check and treated cylinders have been subjected, therefore, have resulted in an alteration in the character of this insoluble potassium combination in the direction of increasing solubility.

The question may now be asked, What significance do aqueous extracts of soils possess with respect to potassium content? Water extracts of soils obtained by displacement, 1:5 water extraction, and percolation methods have played a considerable role in investigations relating to the potassium-supplying power of soils. But, as already indicated (p. 539), the significance of the results needs to be reconsidered in the light of recent acquisitions to our knowledge of the behavior of the adsorbed materials in the presence of electrolytes of which, in soils, calcium is such a dominant factor. Although, as in these experiments, small amounts of potassium are dissolved for long periods of continuous leaching of a soil, this fact affords no indication that the difficultly soluble combinations of potassium would be removed by water in the absence of all traces of electrolytes. A low but effective concentration in electrolytes may be produced by several processes, such as (1) action of the micro-organisms (the soil was not sterile in these leaching experiments), (2) the effect of humic acids which can effect a repartition between the elements adsorbed by the humus-clay complexes and the solution phase in contact with it (*6*), and (3) the effect of the slow solubility of carbonates and of silicic acids in water. Under natural field conditions the potassium fixed by the soil can enter into solution only as the electrolytes are replenished by the processes cited. It follows that the amount of potassium removed in aqueous extraction by any method whatsoever is indicative not only of the amount of "soluble" potassium but also of its temporary content of nitrates, chlorides, bicarbonates, sulphates, etc.

This effect of electrolytes on the solubility of adsorbed potassium is striking. The relationship quantitatively demonstrated by Wiegner and Müller (*23*) with permutite and with six soil types by Barbier (*3*) is, when the exchangeable hydrogen ions are relatively small compared with calcium

$$\frac{\frac{P}{C}}{1+\frac{P}{C}}=\frac{k}{T}\cdot\left(\frac{p}{c}\right)^{\frac{1}{n}}$$

where P and C are the concentrations of potassium and calcium in the adsorbent, p and c the concentration of these elements in the solution, k and n constants, and T the exchange capacity.

This formula shows that at equilibrium the ratio of potassium to calcium fixed in the adsorbing complex determines the ratio of potassium to calcium in solution. Accordingly, for one and the same concentration of potassium in the adsorbing complex at equilibrium, the concentration of potassium in the aqueous phase is never constant as the classical theory of adsorption holds. On the contrary, it is proportional to the final concentration of other cations, of which calcium, because of its relatively greater proportion to other cations in soils, plays the dominant role, and consequently, the concentration of potassium is proportional to the sum p plus c of potassium and calcium dissolved. This sum is equal to the quantity of anions present in the aqueous phase, i. e., to the total salt concentration of the solution. The concentration of the soil solution in potassium is then an increasing function of two factors (1) the ratio $\frac{P}{C}$ between the potassium and calcium "fixed" at equilibrium, which determines the ratio $\frac{p}{c}$ between potassium and calcium dissolved, and (2) the total salt concentration of the solution.

An appreciation of the significance of these facts will explain the difficulties encountered in all attempts to obtain threshold values for soil potassium; and, furthermore, will suggest the direction in which investigations on availability of soil potassium should proceed.

THE SO-CALLED EXCHANGEABLE POTASSIUM

It follows from Wiegner's observations discussed above that the so-called exchangeable potassium is, subject to the limitations of definition of the term "exchangeable", a variable quantity determined by the conditions of the experiment. For the determination of this so-called exchangeable soil potassium, extraction with a solution of either sodium or ammonium acetate has certain advantages over other reagents (*16*). In the present experiments a half-molar solution of ammonium acetate was used. This strength is sufficiently high, especially for percolation methods. Even in one extraction

Barbier (*3*) found that a normal solution of ammonium chloride displaced the whole, and a one-third normal solution 98 percent, of the exchangeable potassium from clay soils.

The results given in table 2 show that under the conditions of continuous leaching adopted in these experiments three extractions with M/2 ammonium acetate were necessary before the concentration of potassium was reduced to that of the constant level reached by the water leachates in the fourth extraction. After the third percolation the amounts of potassium removed by M/2 ammonium acetate were nearly identical for all the soils of this experiment. The addition of the potassium sulphate fertilizer resulted in an increase of the so-called exchangeable potassium of the surface horizons of the treated cylinders approximately two and a half times above that of the check cylinders. The effect of the cover crops on the exchangeable potassium was also quite marked. This is indicated by increases of 50 and 75 percent in the surface and subsurface soils of the check cylinders, respectively, over the corresponding horizons of the original soil.

The residual quantities of potash shown in column 2 of table 6 can be calculated from the amounts of potassium sulphate applied together with that absorbed by the trees from the added potassium sulphate (*19*).

TABLE 6.—*Quantities of residual potash which have accumulated in the exchangeable form in the 0- to 21-inch layer*

Description of soil	Residual K_2O (x)	Excess of exchangeable K_2O of treated cylinders over that of check cylinders (y)	$\frac{y}{x} \times 100$
	Grams	*Grams*	*Percent*
NPK (sod)	508	253	50
NPK (cultivation)	481	266	55
PK (sod)	536	256	48
PK (cultivation)	541	264	49

The differences shown in table 6 between the exchangeable K_2O of the treated cylinders and the corresponding check cylinders of the 0- to 21-inch horizon indicate that about one-half of the potassium applied to the soils may be considered to be in a replaceable form. Actually the amounts must be somewhat lower (p. 541). The remainder, consequently, must have been transformed into a nonexchangeable form and the greater portion in the 0- to 7-inch horizon.

The possibility of the conversion of surface-adsorbed exchangeable bases into a nonexchangeable form and vice versa is suggested by the results of several investigators (*10, 12, 13, 15*). This experiment supplies additional and possibly less vulnerable evidence of the fact.

RELATIVE AMOUNTS OF RESIDUAL APPLIED POTASH REMOVED BY THE DIFFERENT SOLVENTS FROM THE 0- TO 21-INCH LAYER OF THE NPK CYLINDERS

The relative amounts of residual applied potash removed by the different solvents (total of all leachings in each case) can be determined in a manner similar to that used to determine the phosphoric acid removed by various solvents (*21*).

Table 7 gives the potash removed by the different solvents from the 0- to 21-inch horizon of the NPK cylinders expressed as a percentage of the residual applied potash. The amounts of potash dissolved by the unfertilized (check) soils by the respective solvents are subtracted from the quantities removed from the treated cylinders as shown in column 2, under the indicated method of calculation.

TABLE 7.—*Relative magnitudes of the potash removed by the different solvents from the 0- to 21-inch horizon of the NPK cylinders*

IN SOD

Solvent	Indicated method of calculation	Percentage of residual applied K_2O dissolved
	Grams	
Distilled H_2O	$\frac{428.4-225.2}{508} \times 100=$	40.0
M/2 $NH_4.C_2H_3O_2$	$\frac{605.5-350.9}{508} \times 100=$	50.1
0.2 N HNO_3	$\frac{2{,}836.7-2{,}425.2}{508} \times 100=$	81.0

IN CULTIVATION

Solvent	Indicated method of calculation	Percentage of residual applied K_2O dissolved
Distilled H_2O	$\frac{431.2-236.3}{481} \times 100=$	40.5
M/2 $NH_4.C_2H_3O_2$	$\frac{628.3-368.8}{481} \times 100=$	53.9

Distilled water has removed 40 percent; M/2 ammonium acetate 50 to 54 percent, and 0.2 N nitric acid 81 percent, respectively, of the residual potash of the 0- to 21-inch layer. The relatively large percentages removed by distilled water as compared with that removed by M/2 ammonium acetate is related to the phenomenon already fully discussed (p. 541).

Eighty-one percent of the residual applied potash has been removed by 0.2 N nitric acid from the 0- to 21-inch horizon of the NPK cylinder in sod. These values give the relative condition of availability of the potash applied as the fertilizer potassium sulphate.

The potash removed by 0.2 N nitric acid has been designated by Fraps (*9*) as the "active" potash. It is surprising that the exchangeable potash of many soils reported by Fraps is from two to three times the amounts found by him to be soluble in 0.2 N nitric acid. In these cylinder experiments the potash dissolved from the corresponding horizons of the respective cylinders by 0.2 N nitric acid are from two to three times the values for the exchangeable potash even in the first two extractions alone. If the analyses are comparable with those here reported there can be no relation between the replaceable potash and that extracted by 0.2 N nitric acid from different soil types.

THEORETICAL FERTILIZER RATIO OF $N:P_2O_5:K_2O$ FOR APPLE TREES GROWING ON A HAGERSTOWN CLAY LOAM SOIL

It was pointed out in the introduction to this and the two preceding papers of this series (*20, 21*) that the ratio in which nitrogen, phosphoric acid, and potash were absorbed by the NPK- and NP-treated trees was 3.0:0.3:1.5, whereas the ratio in which these same fertilizer entities were applied was 3:8:4. The divergence between these ratios is great.

Certain contemporary German agronomists and horticulturists have sought to determine the fertilizer requirements of crops by modification of the quantities in which the principal fertilizer entities are found in the mature crop considered, by making allowance for the quantities of nitrogen leached and the phosphoric acid and potash fixed by the particular soil used.

The factors most frequently used by the German investigators have been for nitrogen, 0.33; for phosphoric acid, 0.50; and for potash, 0.25. The amount of each of these entities found in the mature crop is then multiplied by the proper factor and the resulting values are added to the amount of each entity in the crop.

Since the quantity of nitrogen in plants is roughly proportional to the supply, this is a rational procedure for determining the nitrogen requirement; but its applicability in determining the amounts of phosphoric acid and potash required is more limited because, owing to fixation, the amounts of these entities absorbed by the plant are not proportional to the supply. In a previous paper of this series (*21*) it was shown that the fixing capacity of any one soil type is a relative and not an absolute property; the amount retained is conditioned not only by the concentration of the phosphate or potassium ions but also by the concentration of other ions, such as hydrogen, silica, iron, aluminum, calcium, magnesium, etc. It is also obvious that the amounts fixed will depend upon the manner of application. By a large localized application of a phosphate or potash fertilizer, the soil in that immediate neighborhood may be completely saturated. It has also been shown in a preceding paper (*21*) that the iron and aluminum of the original soil was only 66 percent saturated with respect to phosphoric acid. At the end of the cylinder experiments, with a residual applied phosphoric acid and potash content of 1,040 and 508 g respectively, one-third and one-fifth, respectively, of this residual phosphoric acid and potash of the NPK cylinders under sod were transformed into very insoluble forms.

The proper values for nitrogen, phosphoric acid, and potash to be used in modifying the amounts absorbed by the trees would therefore become:

Nitrogen leached	0.33×152.3= 50.2 g
Phosphoric acid fixed	0.33×1,040=343.2 g
Potash fixed	0.20×508 =101.6 g

Therefore, the theoretical quantities for application to the NPK cylinder under sod taken as an illustration would be:

Nitrogen	152.3+ 50.2=202.5 g
Phosphoric acid	19.2+343.2=362.4 g
Potash	47.2+101.6=148.8 g

where 152.3, 19.2, and 47.2 are the amounts of nitrogen, phosphoric acid, and potash absorbed by the NPK tree in sod from the added salts (*21*). The theoretical ratio thus derived is 202.5:362.4:148.8, or a 4.47:8.0:3.28 fertilizer. This compares with a ratio used in this experiment based on results in the experimental orchard of 3:8:4.

The reason, therefore, why the ratios in which the nitrogen, phosphoric acid, and potash absorbed by the trees differs so greatly from both the theoretical and "practical" ratios is now apparent.

SUMMARY

The distribution and condition of potash at the end of an experiment lasting 6½ years is given for three horizons of a Hagerstown clay loam soil contained in cylinders planted to apple trees and treated with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate in different combinations.

Approximately one-third of the residual applied potassium has moved into the subsurface (7-to 21-inch) layer. There is no definite evidence of movement of applied potassium into the 21- to 53-inch layer.

Under the conditions of continuous leaching of these experiments the amounts of potash removed from the surface soils of the check and also of the treated cylinders by distilled water was more than half of that removed by M/2 ammonium acetate (the so-called exchangeable potash).

The necessity for the reconsideration of the significance of all water-soluble and so-called exchangeable potash data in soil investigations is discussed.

The system of green manuring adopted in this experiment has resulted in increasing the so-called exchangeable potassium of the 0- to 21-inch layer almost 50 percent.

Only about 50 percent of the residual applied potassium was found in a form soluble in M/2 ammonium acetate. The remainder has been converted into less soluble forms.

The relative quantities of the residual applied potash removed under conditions of continuous percolation from the 0- to 21-inch layer of the NPK-treated cylinder in sod by distilled water, M/2 ammonium acetate, and 0.2 N nitric acid were 40, 50, and 80 percent, respectively.

From the present and earlier reported experiments a theoretical fertilizer ratio for apple trees in a Hagerstown clay loam soil is derived. The theoretical ratio of $N:P_2O_5:K_2O$ is 4.47:8.0:3.28.

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MATHEMATICAL EXPRESSION OF EQUILIBRIUM BETWEEN NITROGEN AND PHOSPHORIC ACID IN PLANTS

It has been known for nearly half a century that phospholipins (phosphatides) found in all cells play a predominant rôle in the physiological processes involved in growth and reproduction. In some plant species phospholipins were found to vary directly with the protein-N and in others with the amide-N.[1]

With the growth of conviction among biologists that the final truth of a phenomenon lies in the mathematical description of it, mathematical expressions for the physiological relationships between the various elements ("entities") and plant response to them have been sought,[2] expressible in terms of simple laws.[3]

In investigations now under way in this laboratory on the influence of the soil and the weather on the action of fertilizers by means of periodic analysis of leaves of the same metabolic age (foliar diagnosis) certain plots of Tier 1, Section B, of the long-continued vegetable fertility plots of the Department of Horticulture, on which potatoes were growing in 1935, have been examined. Plot 15, which has received partly rotted horse manure at the rate of thirty tons per acre, has continuously given the highest yields on this particular tier. In 1935 the yield of tubers was 196 pounds per 1/100th acre plot. The plants growing on this plot, therefore, may be regarded as well nourished and nearing the optimum for the soil and climatic conditions in this region.

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In Fig. 1 are plotted as ordinates and abscissae, respectively, the data for the chemical analysis for total nitrogen (N) and total phosphoric acid (P_2O_5) of the fourth and fifth leaves taken from plants growing on this manure plot (Plot No. 15) and also from three plots treated with commercial fertilizers; namely, nitrogen alone (Plot No. 2), phosphate + potash (Plot No. 8) and nitrogen + phosphate + potash (Plot No. 10). The numerals 1, 2, 3, 4 indicate, respectively, the coordinate points for each of the sampling dates—July 7, July 29, August 9 and August 24, 1935.

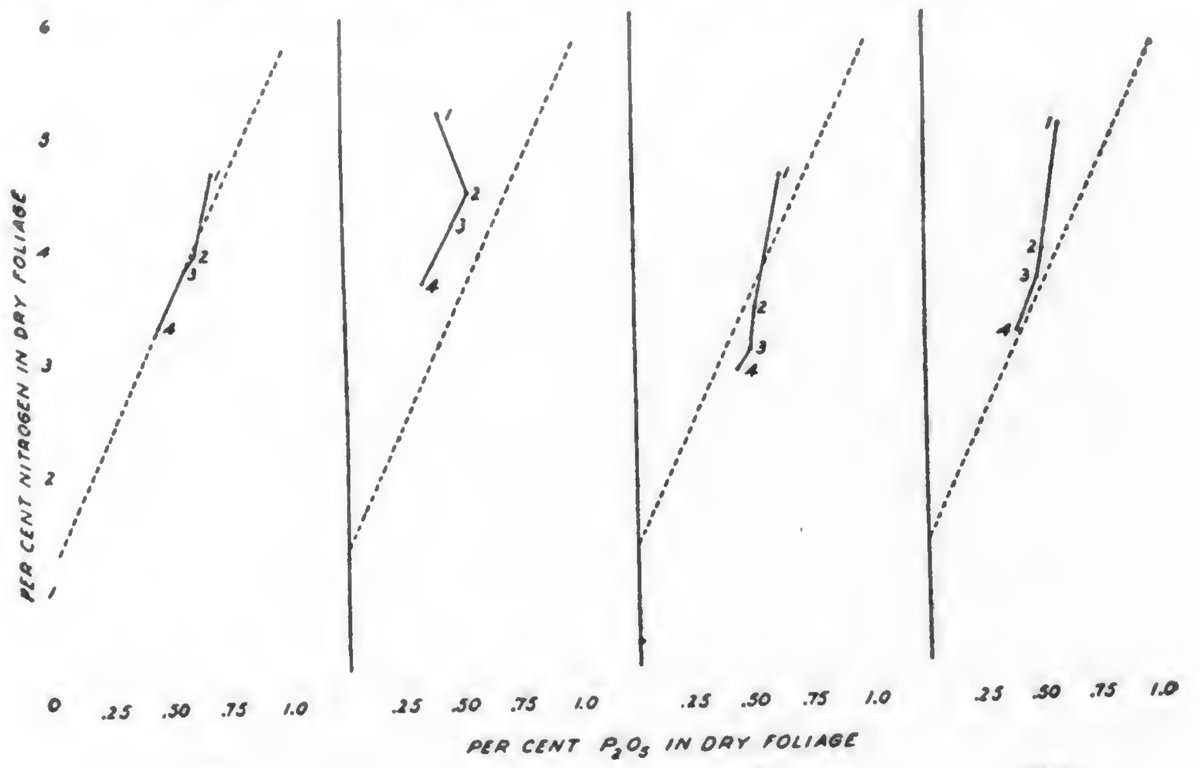

FIG. 1. Showing the deviations from the optimum physiological balance between nitrogen and phosphoric acid in four differently treated plots.

The broken line has been drawn by joining the coordinates (given in columns 1 and 2, Table 1) for the third and fourth samplings, taken on August 9 and August 24, respectively, of leaves of the same physiological age of plants growing on the manure plot. The scale for P_2O_5 is twice that for N. *The equation to this line is* $y = 2.2x + 1.31$. The ordinates of this line corresponding to the amounts of P_2O_5 found by analysis at successive sampling dates vary very little from the amounts of N found experimentally. The deviation of experimental values calculated from the above equation is shown in the last column of Table 1.

TABLE 1

EXPERIMENTAL VALUES FOR P_2O_5 AND EXPERIMENTAL AND CALCULATED VALUES FOR N IN DRY FOLIAGE AT FOUR SAMPLING DATES FOR PLANTS GROWING ON THE MANURE PLOT

Dates of sampling	Experimental values		Calculated values	Relative deviation
	P_2O_5 (E_x)	N (E_y)	N (E_{y_1})	$\frac{E_y - E_{y_1}}{E_y}$
	Per cent.	Per cent.	Per cent.	Per cent.
July 7 . . .	0.688	4.650	4.340	+ 0.067
July 29 . . .	0.608	3.920	3.980	– 0.015
Aug. 9 . . .	0.564	3.790	3.790	0
Aug. 24 . . .	0.448	3.280	3.280	0

On the first sampling date (July 7), nitrogen in this manure plot is somewhat in excess in relation to the optimum value; a result which can be accounted for by an excess absorption of nitrogen caused by the intervention of nitrogen produced by nitrification in a favorable season from the cover crops used in the rotational system. On the second (July 29), third (August 9) and fourth (August 24) sampling dates equilibrium between N and P_2O_5 is attained.

The yields of tubers from the three other plots are: N (Plot No. 2) 109 pounds, PK (Plot No. 8) 148 pounds and NPK (Plot No. 10) 163 pounds per 1/100th acre plot. The deviation from the optimum (broken) line with respect to position, form and length between sampling dates shows in each case the nature of the disequilibrium between N and P_2O_5 in the plants growing on the particular plot and may be regarded as an indicator of the lack of physiological balance between nitrogen and phosphorus. Details will be published elsewhere.

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RECENT TRENDS IN VITAMIN RESEARCH

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IN DISCUSSING the topic assigned to me, I have made no attempt to make a complete survey of recent literature. I have attempted to stress representative types of research work of the past two or three years and to emphasize certain types of investigations which have assumed importance in connection with our own research program at the Pennsylvania State College.

Carotene and vitamin A. Since the discovery that beta carotene is the precursor of vitamin A, nutrition workers have been devoting considerable time and attention to the establishment of carotene as an international reference standard for the assay of vitamin A. As a result, three types of research activity have been stimulated with the hope that all chemical and biological variables could be ascertained. These research activities can be classified as follows: (a) methods of isolation and purification of beta carotene, (b) feeding technique studies accompanied by chemical and physical measurements with the view of establishing recommendations for unitage, and (c) studies which have led to a better understanding of physiological functions of carotene.

The American committee, working at the request of the revision committee of the United States Pharmacopoeia, has coöperated with the League of Nations committee, and many laboratories in many countries have been studying various phases of this question.

One of the first problems which confronted workers in this field was the selection of a suitable solvent, for it soon became evident that some solvents were better than others. Workers in my laboratory (1) soon ascertained that mineral oil could not be used as a solvent for carotene, owing to the fact that this oil is not absorbed in the digestive tract; and since it possesses a preferential solvent effect on carotene, the latter is excreted with the unabsorbed mineral oil, thus preventing carotene utilization. It also became evident that carotene was not stable in all solvents (2), with the result that several laboratories made a study of this question, using biological response, color comparisons, and light absorption studies with various solvents under various conditions. It was found that solvents could be arranged in the following order so far as their superiority is concerned in stabilizing beta carotene: cottonseed oil, sesame oil, coconut oil, olive oil, corn oil, and wheat-germ oil. It was found that it was impossible to use esters such as ethyl laurate owing to the fact that carotene was unusually labile in this type of solvent. It was found that additions of hydroquinone improved the keeping qualities of carotene when ethyl laurate was used as the solvent. Carotene is unstable in the light, and it has become the practice to keep carotene preparations in the dark and preferably in a cool place. Coconut oil has been a keen competitor of cottonseed oil for the introduction of carotene into food and pharmaceutical preparations. At present the international reference samples are sent to workers over the world in small vials using coconut oil as the solvent and hydroquinone as the preservative.

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[1] Presented before the food and nutrition division of the American Home Economics Association, Seattle, Washington, July 9, 1936.

The first samples of carotene were collected from a number of laboratories over the world in 1931. These were sent to London, England, where they were all pooled into one large sample which was recrystallized and purified. This was called the "international sample." It is now evident that this sample could not have been pure because 2.5 gamma to 5 gamma were required to produce the growth response required by the Sherman standard and to prevent xerophthalmia. As purification methods improved and methods were devised for the separation of the isomeric carotenes, the potency increased. Today crystalline beta carotene is available which prevents xerophthalmia and produces the slow growth required by the Sherman standard at doses as low as 0.5 gamma.

Workers have reported varied successes with the application of light absorption technique on the determination of vitamin A content of miscellaneous oils. English workers, led by the Lister Institute, and the British pharmaceutical interests have made a rather thorough study of this problem. If all oils are saponified under standard conditions, if the unsaponifiable residues are dissolved in specified solvents such as cyclohexane, etc., it was found that light data could be used in a practical way and translated into biological units by using a factor to convert the extinction coefficient at 328 millimicrons to biological units (international). The conversion factor is, however, considered provisional.

A few interesting and suggestive studies have been made relative to the absorption and utilization of carotene and vitamin A. The work of Drummond and his associates (3) shows quite clearly that vitamin A exists in the lymph as an ester of the fatty acids. It is their suggestion that proper and efficient absorption of vitamin A is dependent upon esterification during or preceding absorption. Neither vitamin A nor carotene could be made to pass a dialyzing membrane, indicating that these substances exist in colloidal form.

Greaves and Schmidt (4), working with rats, suggest that the passage of carotene through the intestinal wall depends on the presence of bile acids, while the preformed vitamin A can be absorbed without the aid of bile acids or other bile ingredients.

Davies and Moore (5) have fed massive doses of vitamin A to rats and have studied the speed with which this vitamin is lost, following relatively enormous liver storage. It is interesting to note that superfluous liver reserves are lost relatively rapidly compared with those reserves that are known to satisfy the normal physiological requirement. When carotene was fed this was not the case, and no explanation is as yet available for this apparent discrepancy in results.

Ralli, Pariente, and their co-workers (6) have studied blood carotene in normal and diabetic individuals. The same doses of carotene in oil were administered orally to four normal and four diabetic individuals for a period of four months. Carotene rose to a higher point in the blood of diabetic patients and diminished at a slower rate after feeding. They interpret this to mean that the liver in diabetes has lost some of its power to transform carotene into vitamin A and that storage of carotene in the liver tends to delay absorption of blood carotene.

Dr. Florence Tabor (7), working in my laboratory, has studied the effect of vitamin A (carotene) intake on the vitamin D requirement of rats in the production and cure of rickets. She concluded that relatively large doses of carotene (90 international units per gram of food) tended to increase the severity of rickets during the depletion period, while there was a tendency for this amount of carotene slightly to retard recalcification during the curative period. She found that line-test and X-ray measurements gave the most accurate and dependable results, while bone ash and blood serum methods were less dependable. She places the greatest confidence in line-test values.

Sutton, Setterfield, and Krauss (8) have studied the influence of vitamin A deficiency on the degeneration of nervous tissue in the rat. They observed a degeneration of the myelin sheath of peripheral nerves, which occurs about the same time as xerophthalmia, and found that the degeneration had become extensive before symptoms of paralysis could be noted. Adequate doses of vitamin A arrested the degenerative changes but did not relieve external symptoms once they had appeared. These authors suggest that the typical metaplasia observed in vitamin A deficiency may be due to similar changes in the phospholipids of epithelial cells, resulting in a disturbance of normal cell permeability.

Zimmermann and Cowgill (9) have made a similar study in which they describe a nervous disorder characterized by muscular weakness, in-co-ordination, and, in severe cases, paralysis of the posterior extremities. They observed demyelination of the peripheral nerves, particularly the sciatics. When the ration was supplemented with carotene this disorder did not develop. When carotene was added early in the experimental period animals were restored to good health. When the neurologic disorder was allowed to become severe it was impossible to obtain relief by carotene administration.

In spite of many clinical publications to the contrary, papers still continue to appear relative to the influence of vitamin A on resistance to colds and respiratory infections. Miss Cameron (10) of West Virginia could not prove that cod-liver oil, halibut-liver oil, or carotene reduced the average number of colds in a group of 200 students from October to April. She did conclude, however, that the average duration of colds was reduced as a result of vitamin A therapy. She emphasizes the fact that vitamin A is not a specific against colds, but states that it is a factor in reducing severity and duration of colds. It is necessary, therefore, to sound the warning and make the point clear to the lay public that vitamin A cannot be considered a cure-all for colds.

Dr. Redding (11) of Scranton, Pennsylvania, advances arguments to prove that phlyctenular disease symptoms in humans and in rats are quite similar, as indicated by clinical and microscopic findings. He believes that this disease (which is sometimes known as conjunctivitis lymphaticus or conjunctivitis exzematosa) is due to vitamin A deficiency and advocates larger doses of cod-liver oil as an aid in its treatment.

A number of dairy research workers describe a type of blindness in cattle which seems to be a form of vitamin A deficiency.

Maynard and his co-workers (12) have continued their work on the production of muscular dystrophy in farm animals produced by cod-liver oil feeding. While it is possible to demonstrate muscular dystrophy in sheep and goats, recent results indicate no gross evidence of dystrophy in calves. They state that calves, if affected at all, are much less susceptible to cod-liver oil injury. It is premature, therefore, to anticipate a possibility of cod-liver oil injury in humans until more data are forthcoming.

Papers continue to appear relative to the effect of diet on the carotene and vitamin A of cow's milk. It is generally agreed that goats and sheep possess a marked ability to transform carotene into vitamin A. Guernsey and Jersey breeds might be considered pathological in this respect, going through life in a more or less continuous state of carotenemia. Our work at the Pennsylvania State College indicates that this is true, and we find no difference in the total biological value per gram of butter fat from the various breeds; but since Jersey and Guernsey milk contain more butter fat, it stands to reason that these milks will usually contain more vitamin A units per quart, unless the milks are standardized to a uniform fat content.

Meigs and Converse (13) of the United States Department of Agriculture advance evidence to prove that vitamin A (carotene) plays an important role in the production of satisfactory reproduction and lactation in dairy cattle. Wiseman, Kane, and Cary (14), working in the same laboratory, have studied the stability of carotene in baled alfalfa hay. Carotene destruction was found to be a function of temperature with a minimum loss of 2.6 per cent per month during the colder months (40°F.) to 6.5 per cent per month during the warmer months.

The B vitamins. I hesitate to discuss the newer developments so far as research work in the B complex field is concerned, because opinions are changing so rapidly. Suggestions have been made relative to the existence of B_1, B_2, B_3, B_4, B_5, B_6, H, Y, and so on. In my opinion, we can delay discussion of B_3, B_4, and B_5 until we have more satisfactory proof of their existence and of their clinical importance.

So far as my own interests are concerned, the outstanding development in regard to B_1 is the brilliant work of Williams and associates in this country and of English and German workers in the production of crystalline vitamin B_1 and the determination of its structure (15, 16, 17, 18, 19, 20, 21). Not only is it now available in limited quantities for experimental work with laboratory animals, but since it has been synthesized in the laboratory the time may not be far off when the pure artificial product may be available at a lower cost.

Most workers are now agreed that Williams' formula is essentially correct and that vitamin B_1 consists of a dimethyl-amino-pyrimidine nucleus attached to a 4-methyl-5-β-hydroxy ethyl-thiazole. The crystalline product has been used successfully by a few clinical workers.

I shall pass over a discussion of the work being done relative to the adoption of an accepted international assay technique, since referee committees are now at work in an attempt to arrive at a type of technique that will meet with general acceptance. Whether the official method (U. S. P.) will eventually be a pigeon method or a rat method is undecided. Whether growth, maintenance, feeding, or injection methods are preferable is still open to question. It would appear that crystalline B_1 will be adopted as the international standard to replace the acid clay adsorbate, but nothing further has been done so far as I am aware.

Time will not permit a discussion of recent studies using the newer techniques, but I might add in closing my discussion of vitamin B_1 that Dr. Morgan and her co-workers (22) at California find that vitamin B_1 is not lost in appreciable amounts during the baking of bread and that the size of the loaf has some influence on the destruction of this vitamin. Less destruction seems to take place in the larger loaves. She states also that wheat germ has three times as much vitamin B (vitamin B_2) as eggs, five to six times as much as cow's milk, and two-thirds as much as beef liver.

Dr. Kuhn of Heidelberg University has isolated flavin (d-riboflavin) from liver, milk, and eggs. Kuhn and some other European workers tend to call this pigment "B_2" because of its growth-stimulating properties. Some workers have ascribed dermatitis-curing properties to lactoflavin, but other workers (including ourselves) cannot find that this is always the case. We obtain a growth response, but the florid type of skin lesions do not respond to flavin additions to the diet.

György (23), publishing from Harris' Laboratory at Cambridge University, England, seems to have irrefutable evidence that the true anti-dermatitis factor is a heat stable fraction (probably identical with Chick's "*Y*" factor) which he calls B_6. He feels that this factor plus flavin forms a combination which is biologically equivalent to the old G or B_2. Dr. Guerrant, in my laboratory, has data which seem to support this point of view, and we are inclined to question the existence of the B_4 factor discussed by Kuhn, Elvehjem, and other workers. Fish muscle seems to be one of the best sources of B_6, and it is interesting to note that fish muscle is usually almost devoid of B_1. György believes the type of rat dermatitis to be similar to acrodynia in infants and raises the question as to whether rat dermatitis is similar to or identical with human pellagra.

Dann (24) of Duke University goes further in this regard and points out that maize, which is the principal food of pellagrins (and, therefore, the principal pellagra producing food), contains relatively large amounts of vitamin B_6, which cures rat dermatitis readily. Furthermore, Dann points out that human pellagra depends on action of light on the skin to bring out the characteristic bilaterally symmetrical dermatitis. This, he finds, is not the case with rats, since he can produce rat dermatitis with relative ease in the dark. However, recent experiments by Dr. Guerrant lead us to believe that it is possible to produce a bilaterally symmetrical type of rat dermatitis which seems to be similar to if not identical with that which characterizes human pellagra.

The chemistry of the flavin molecule has been elucidated by Karrer, Kuhn, Stern, Von Euler and co-workers (25), and their conclusions have been confirmed by laboratory synthesis. Lactoflavin is now definitely recognized as 6,7-dimethyl ribityl isoalloxazin and is sometimes referred to as a riboflavin.

A large number of related compounds have been synthesized by substitution in the isoalloxazin nucleus or by varying the configuration of the carbohydrate side chain. Flavin has been isolated from Warburg's "yellow enzyme" and Warburg, Kuhn, Theorell, and Von Euler have indulged in a series of interesting theoretical discussions relative to the possible interrelationship of vitamins, enzymes, and hormones. Flavin is readily inactivated by light but is protected from light inactivation by oxygen.

Dr. Guerrant (26), in my laboratory, has studied the influence of the type of carbohydrate on the synthesis of vitamins B and G in the digestive tract of rats which practice coprophagy. His results indicate that sucrose, glucose, and starch are usually assimilated sufficiently rapidly so that these carbohydrates rarely reach the cecum in appreciable amounts. Dextrin, on the other hand, is digested and assimilated at a slower rate, with the result that this carbohydrate reaches the cecum where organisms (probably yeasts) have an opportunity to multiply. These organisms synthesize the B and G complex, which is not readily assimilated in the lower bowel but is voided in the feces. Lactose occupies an intermediate position between the soluble sugars and dextrin. When feces are reingested by coprophagous rats, sufficient amounts of vitamins B and G are absorbed to supply the needs of the rat for indefinite periods. It is necessary, therefore, to keep these facts in mind in assaying foods for vitamins B and G.

Hogan and Richardson (27, 28) describe experiments in which rats are denuded and are caused to develop dermatitis on a diet devoid of G but containing B (B_1) in the form of tikitiki. Flavin additions produced a new growth of hair but had no effect on the florid type of dermatitis. Alcoholic extracts of starch or small doses of wheat-germ oil brought about complete healing of the skin lesions. Wheat-germ oil, however, had no effect on relieving the denuded condition. It is clear, therefore, that we must proceed with caution before we will be in a position to recommend definite standards for the assay of the various fractions of the old B complex.

Vitamin C. After discussing the conflicting data and opinions relative to the B complex, it is a relief to come to vitamin C. This vitamin is specifically an antiscorbutic factor; hemorrhagic and dental responses are consistent and orderly; the chemistry of vitamin C is relatively simple and understandable; and this vitamin can now be assayed with reasonable accuracy by chemical means (29).

As a result of the development of titration methods for vitamin C (ascorbic acid), a relatively voluminous literature has appeared regarding the distribution of this factor in foods and tissues. The fact that vitamin C is found in so many growing tissues has led to the belief that it may be of considerable importance in vital processes. Cambridge University workers have shown that ascorbic acid appears simultaneously with vital reproductive changes when eggs are incubated and when seeds are germinated.

As a result of the fact that ascorbic acid is very labile, being reversibly oxidized and reduced, evidence is accumulating which indicates that this vitamin may play an important role in certain enzyme systems. It seems clear that ascorbic acid plays a part in hydrogen transfer during tissue respiration and may act as an activator of molecular oxygen.

Ascorbic acid exists almost entirely in reduced form in living tissue and, with the exception of citrus fruits, seems to be highest in those types of tissues characterized by high metabolic activity. It is found in most animal tissues and is highest in glandular tissues and lower in adipose and muscular tissues. Oxygen uptake is lowered in vitamin C deficiency, and its absence brings about characteristic changes in teeth, bones, and blood-vessel walls.

It can be synthesized by birds and rats, and its distribution in the tissues is the same as that of those animals which must obtain this factor from the food supply. The precursor or precursors of ascorbic acid in rats and chickens is a matter of conjecture, although Guha and Ghosh have expressed the opinion that mannose is one of the precursors. We are now also investigating this phase of the problem in our laboratory.

Considerable evidence exists (starting with the work of Dr. A. F. Hess) to show that there is a loss of resistance to certain types of infection when the ascorbic acid supply is low. Rinehart (30) of California contends that a form of rheumatic fever usually develops in guinea pigs as a result of infections which accompany a vitamin-C-deficient diet.

Latest work on the stability of vitamin C in foods adds very little to what we have already known. Many environmental factors are unfavorable, such as light, high temperatures, oxidation, contact with copper and certain other metals, liberation of oxidative enzymes by tissue disorganization. It is of interest to know that many of the better canning organizations now produce canned goods which compare favorably in vitamin C content with that of the fresh food materials from which they were made.

Reports from all over the world indicate that we still have a vitamin-C-deficiency problem. Blood fragility tests, urine titrations, and other methods are offering new possibilities for diagnosis of vitamin C deficiency. No present method is completely satisfactory. Toxic substances and pathological disturbances may affect capillary resistance, leading to incorrect diagnosis of incipient scurvy. Individuals vary in their storage of vitamin C, and it is not possible to excrete this vitamin in the urine until the tissues are saturated. This may account for the fact that differences of opinion exist relative to the effect of the vitamin C content of the diet on the vitamin C content of cow's milk.

Dr. Guerrant, Dr. Bechdel, and their students (31) at the Pennsylvania State College have just finished a study of the influence of breed characteristics and stage of lactation on the vitamin C content of cow's milk. Of five breeds studied, milk from the Brown Swiss breed contained the most ascorbic acid, while milk from Holstein cattle contained the least. These differences, although significant, were not marked. Stage of lactation had a definite influence on the ascorbic acid content of milk, this vitamin being high in the early stages of lactation, decreasing to a minimum in two months, and then increasing to a maximum in the latter stages of lactation. These fluctuations could be correlated with milk production, indicating a rather constant excretion of ascorbic acid, the concentration being higher at periods of low production and lower at periods of high milk production.

Selleg and King (32) of the University of Pittsburgh, have studied the ascorbic acid content of human milk. They found that human milk had about four to five times as much vitamin C (ascorbic acid) as cow's milk and that mother's milk can be made richer in this vitamin by feeding orange juice, providing the mother's diet has been deficient in this regard. If, however, the vitamin C intake has been high, it is impossible to increase the vitamin C content of the milk above the optimum level of the individual. These authors conclude that an infant should receive an average of 50 mg. per day of ascorbic acid during the nursing period.

Vitamin D and rickets. The conviction has been growing that we are confronted with the problem of recognizing more than one vitamin D. Researches of Bills, Koch, Waddell, and their co-workers (33) point to the fact that cholesterol or some product of cholesterol is activatable with ultraviolet light and that this substance is not identical with ergosterol in physiological action. All researches to date indicate that the activated cholesterol preparations, when fed to chickens, possess physiological properties which seem to be identical with those possessed by the natural vitamin D of cod-liver oil.

Cholesterol can also be activated by chemical means. Bills and McDonald have obtained an active antirachitic product by heating cholesterol with fuller's earth and have advanced the opinion that more than one antirachitic isomer of cholesterol may exist. Yoder has made a sulfonated product from cholesterol, after dehydration, which possesses antirachitic potency. Other cholesterol and ergosterol derivatives have been shown to be activatable.

Bills expresses the opinion that at least seven forms of vitamin D exist and that it is theoretically possible to postulate a great number of molecular configurations which may possess antirachitic properties.

Dr. P. C. Jeans (34) of the University of Iowa has just published an interesting résumé of all clinical literature to date which has to do with vitamin D requirements of humans. He recognizes two forms of vitamin D, that from animal sources and that from plant sources. He emphasizes the difficulty of correlating rat data with human demands and stresses the danger of restricting the use of vitamin D solely for recalcification, particularly as measured by clinical observations. While X-ray methods are useful, he feels they do not give information regarding optimal calcium retention and other conditions.

In discussing the clinical value of vitamin D milks he stresses the point that calcium retentions in infants are variable and that best results are obtained when the daily intake is around 400 international units per quart, which is roughly equivalent to one teaspoonful of average cod-liver oil (350 international units).

Dr. Jeans states that irradiated milk is satisfactory but that it probably usually contains about the threshold amount of vitamin D necessary for calcium retention and growth, namely, 95 to 100 international units. He also discusses such products as yeast milk or metabolized milk, cod-liver oil (concentrate) milk, activated ergosterol milk. He further states that cod-liver oil milk and irradiated milk seem to be equal in potency, unit for unit, while metabolized milks and ergosterol milks seem to be of equal antirachitic value. He feels that the differences existing between the two classes of milk are not great and suggests a ratio of 1.5 to 1.0, classifying all milks containing animal sources of D in one group and all milks containing vegetable sources of D in the other. He lays considerable emphasis on the necessity of feeding 400 international units of vitamin D per day to insure a sufficient supply for proper calcification, calcium retention, and growth. Dr. Jeans concludes his excellent résumé of the literature with the statement that no data are available to determine directly the minimum rickets-preventing dose of vegetable-source vitamin D or the amount of this factor which permits good growth and proper calcium retention.

Vitamin E. I know of no outstanding developments in the vitamin E research field for the past two years which merit emphasis here beyond the statement that considerable progress is being made in the chemical study of this antisterility factor. Evans and co-workers, Askew, Olcott, and Drummond, have published a number of papers regarding such subjects as the methods of isolation, the physical and chemical properties of potent fractions; but no one has arrived at definite conclusions regarding its structure other than to suggest that it may be an unsaturated sterol. Emerson, Emerson, and Evans (35, 36) have recently announced the isolation of an alcohol which appears to be similar to, if not identical with, vitamin E so far as physiological properties are concerned.

Evans and Lepkovsky (37, 38) have continued their interesting work on fat-free diets. They describe a type of sterility which apparently is not identical with vitamin E sterility and a type of dermatitis which is evidently a characteristic fat-deficiency disease. It is possible to prevent and cure the disease with small amounts of unsaturated fatty acids of the type of linoleic and linolenic acid. The term "vitamin F" has been used in connection with these unsaturated fatty acids, but the designation has not yet received scientific recognition.

It would appear, therefore, that the predominating trends in vitamin research at present (as in the recent past) are in the direction of the isolation of vitamins and the determination of configuration followed by laboratory synthesis; the improvement and standardization of assay techniques and of vitamin unitage; the re-estimation of vitamin content of foods, vitamin stability during food processing, and so on, using the newer techniques; and further elucidation of the vital physiological functions of vitamins in the living organism.

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'BURN' OF TOBACCO IMPORTANT FACTOR IN VALUE OF LEAF

Haley Offers Tips On Quality; Warns Against Muriate Of Potash

Dr. D. E. Haley, professor of Soil and Phytochemistry at the Pennsylvania State College, has prepared an article expressly for publication in the Lancaster newspapers for the benefit of Lancaster county tobacco growers, who produce more than 90 per cent of all the tobacco grown in the state.

The article, entitled "Muriate of Potash and its Relation to the Burning Quality of Pennsylvania Cigar-Leaf Tobacco," follows in full:

The citizens of Pennsylvania have reason to be proud of its tobacco industry, which is largely centered in Lancaster county, and to congratulate those who have been responsible for its progress to the high position it maintains today. There is however, plenty of opportunity for its advancement. This may be accomplished if the tobacco grower appreciates the significance of those factors which impair or improve the burning quality of his crop and upon which its value so largely depends.

According to Dr. W. W. Garner, one of the early research workers in this field, burning quality includes the fire-holding capacity or the length of time the tobacco will continue to glow when ignited, the evenness and completeness of combustion, and the character of the ash.

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The ideal conditions with respect to burning quality appears to be obtained when the tobacco holds fire well so that the smoker is not annoyed by the need of either smoking rapidly or steadily; when the tobacco burns evenly, thereby reducing the burning surface to a minimum; and when the burn is sufficiently complete to leave a light, coherent ash, indicating good combustion, leaving in the smoke only those products which impart to it the desirable aroma and pungency.

While the tobacco grower is concerned primarily with yield, he appreciates that the sale of his product largely depends upon its burning quality. Furthermore, he realizes that increased cigar consumption lies mainly in the betterment of the manufactured product. This is largely influenced by the manner in which the tobacco is handled in the field and curing shed before it enters the manufacturing plant. The importance of the burning quality of Pennsylvania tobacco has been appreciated by growers, research workers. and cigar manufacturers. As a result, considerable progress has been made in fertilizer treatment, which affects the yield and quality of the crop. A number of factors are involved in the production of good burning tobacco, but the role of potash in the fertilizer mixture is of paramount importance. Experiments have shown that where potash has been present in suitable quantities, and in the proper form, there results, among other things, a thinner. broader leaf of better burning quality than where potash is lacking. For this condition to be obtained, potash must exceed the quantities of the other plant food elements present in the fertilizer. This is true whether manure is or is not applied with the fertilizer.

In order for potash to function properly it must be in such a condition as to react with certain compounds of the plant. Potash from the soil minerals meets this condition, as it is absorbed from such a medium in a form closely approximating carbonate of potash. The use of carbonate of potash as a tobacco fertilizer in the form of cotton hull ashes, therefore, is fundamentally sound, although other forms may prove equally efficient if not more so. Nitrate of potash is satisfactory as a carrier of potash, although sulfate of potash is quite satisfactory and is more commonly used.

Muriate of potash is entirely unsatisfactory as a carrier of potash for tobacco. The injurious effects of muriate have long been recognized by the tobacco growers of Lancaster county. There is now a state law prohibiting the use of muriate of potash in brands of tobacco fertilizers; this law has met with approval. In sipte of this law, however, cases have been known in recent years where muriate of potash has been applied to the tobacco crop, not as a constituent of a tobacco fertilizer but as a constituent of certain other brands. These were but isolated cases, however, and are not representative of the usual cultural practices.

While the tobacco grower rarely uses muriate of potash as a tobacco fertilizer, in many instances he uses it indirectly. The common practice is to follow corn or legumes with tobacco, but cases are common when tobacco is allowed to follow a crop of potatoes or tomatoes. If muriate of potash has been used for these crops they should not be followed by tobacco. Experiments have shown that muriate of potash has been carried over in the soil for at least a year, and in quantities sufficient to injure the burning quality of the tobacco grown on these soils.

The fertilizer manufacturers have realized this condition and brands of potato and tomato fertilizers are on the market in which the potash is carried as sulfate. The tobacco grower would do well to use such a carrier on potatoes or tomatoes, if tobacco follows these crops, or if muriate of potash is used, to see that tobacco does not follow them

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From the (Lancaster, Pa.) Intelligencer Journal, February 3, 1937

Fertilizers for Pennsylvania Cigar-Leaf Tobacco*

By D. E. Haley, Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pennsylvania

SEVERAL years ago I was one of several individuals who were asked to appear before a group of fertilizer manufacturers and their representatives meeting in Lancaster, Pennsylvania, to discuss the question of a rational fertilizer for Pennsylvania cigar-leaf tobacco. It was hoped that the different manufacturers might formulate a mixture which, in the opinion of the State and Federal workers, would meet the average conditions under which tobacco is grown as to yield, and also satisfy the demands of the cigar manufacturer as to quality. Realizing the impossibility of choosing a single formula which would meet all conditions in Lancaster County, which is the center of the cigar-leaf industry of Pennsylvania, we recommended that a 3-5-12 mixture be used at the rate of 1,000 pounds per acre, with approximately 10 tons of manure.

We had good reasons for making this recommendation. For generations most of the tobacco soils involved had received liberal applications of stable manure. This was applied immediately before the tobacco crop, which, in many cases, received no other fertilizer. Under such conditions, the effect of nitrogen overshadowed the other necessary fertilizing elements, hence a crop was produced that was quite susceptible to various diseases in the field, and failed to meet the demands of the cigar manufacturer from the standpoint of burning quality. Therefore, we called for a fertilizer having a low nitrogen content.

Part of the three units of nitrogen was to be nitrate of soda, although nitrate of potash might have proved more satisfactory. The remainder was to be from cottonseed meal. This mixture provides a readily available form of nitrogen for the young plant. Cottonseed meal has always proved desirable as a carrier of part of the nitrogen for tobacco.

We recommended what was thought was the absolute minimum of phosphoric acid, knowing that in the majority of cases a higher quantity might be used to advantage. Notwithstanding the fact that the phosphoric acid content of Lancaster County tobacco soils is quite low, the farmer is loath to use any more than a bare minimum, because of the cost. This holds for the other constituents as well.

We recommend what appears to be the maximum quantity, or twelve units of potash. This is a limiting factor in many of these soils. Owing to the high lime content, the availability of this element appears to be reduced to a minimum. An adequate supply of potash insures increased resistance to disease and aids in the retention of water by the plant through the formation of cutin.

The chief reason for the recommendation on potash, however, was that this element enhances the burning quality of tobacco. It is the opposite of nitrogen in this respect, and satisfies the most outstanding demand of the cigar manufacturer, who is able to manufacture a product which will meet the approval of the smoker if the tobacco possesses good burning qualities.

Our recommendations found ready acceptance. The manufacturers immediately placed a 3-5-12 mixture on the market. During the 1935 and 1936 growing seasons I visited about 500 representative farms in different parts of Lancaster County. In many cases manure alone was still being used for tobacco. Manure is a very good source of organic matter, and I believe that the chances of producing a good crop are more nearly insured by its presence than by its absence. In no case, however, have I noted a crop of good quality where manure alone was used. During the 1936 season we obtained rather striking results by reenforcing manure with a 2-4-12 fertilizer applied at the rate of 1,000 pounds per acre where the availability of phosphorus, as measured by the so-called "quick tests", was relatively high.

I was surprised to note that in some instances the only application made to the field was ground limestone, and this in spite of the facts that these soils were alkaline in reaction.

In some cases the 3-5-12 mixture was used without manure, although it was never recommended by us that it be used in this way. In a majority of cases, only small quantities of this mixture were used—200, 250, 300 or 400 pounds per acre. Since not less than 50 pounds of phosphoric acid should be applied, such small quantities of this mixture would exercise very little influence on the yield, and perhaps on the quality also. In the few instances where the 3-5-12 fertilizer was used in the way it was intended, very favorable results were obtained.

In our field experiments at Roseville, we have been able to produce excellent quality and yield without the use of stable manure, using a 6-8-12 fertilizer at the rate of 1,000 pounds per acre. In this mixture one-third of the nitrogen is supplied as nitrate of soda and the remainder as cottonseed meal. The phosphoric acid is supplied as 16 per cent superphosphate and the potash as sulphate of potash. In every instance better results were obtained when this fertilizer was applied to the row one week prior to transplanting, rather than broadcast. Excellent results have been obtained by the reenforcement of manure with a 3-8-12 fertilizer applied at the rate of 1,000 pounds per acre.

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* Paper presented before the Sales Meeting of The National Fertilizer Association in Philadelphia, January 18, 1937.

Improvement in the tobacco industry will only come when there is close cooperation between research workers, the cigar manufacturers, those who are interested in the manufacture and sale of fertilizers, and tobacco growers. It will be futile for us to say what constitutes a good tobacco fertilizer if the growers will not use it and in the proper quantity. There has been an increased demand in recent years for cigars containing Pennsylvania tobacco, but the continuance of this demand will depend in a large measure on quality. Under normal conditions quality appears to be controlled by the tobacco grower himself, especially by his attitude towards the fertilizing of the crop. With this in mind, I will discuss a few of the important angles of this problem.

The Use of Manure

Manure has been used largely in the past and will continue to be used. The use of manure should not be discouraged. It is an unbalanced fertilizer at best and should be reenforced, especially with phosphoric acid and potash. The reversion of phosphoric acid is retarded by its use; this is an advantage. Neither should we minimize the effect it has on the physical condition and water-holding power of the soil. The Pennsylvania tobacco grower has an advantage over the Puerto Rican grower, since the latter has no cheap source of organic matter and must rely on mineral fertilizers alone. When and if, however, we succeed in divorcing the grower from the idea of using manure alone, an important step will have been taken in the right direction.

The Use of Phosphoric Acid

I question the wisdom of adding less than 50 or more than 80 pounds of phosphoric acid per acre. Examination of our Lancaster County soils shows a lack of available phosphoric acid except where annual applications of organic materials have been made. Care should be taken, however, to properly balance this constituent with respect to nitrogen and potash.

The Use of Nitrogen

The tobacco plant requires large quantities of nitrogen, but there is danger of supplying too much of this element. During 1935 we treated some plots with a 9-8-6 fertilizer applied at the rate of 1,000 pounds per acre. The result was that the burning quality was reduced to a minimum but compared favorably with plants receiving manure alone. Even when the constituents are better balanced, our results show that in the majority of cases no more than 60 pounds of nitrogen to the acre need be added. This may be reduced to a minimum in some cases. The greatest quantity of nitrogen found in tobacco grown on our three-year rotation plots was from a plot receiving 0-8-12 fertilizer, applied at the rate of 1,000 pounds per acre. In this instance, however, alfalfa preceded tobacco. Plowing under clover also enhances the nitrogen content of the crop.

The question of too much nitrogen is one of our most serious problems. This condition appears to be paralleled to Puerto Rico, where conditions favor the fixation of atmospheric nitrogen, since bacteria may work at an optimum temperature throughout the entire year. The most common fertilizer used there is a 6-7-8 mixture applied at the rate of 800 pounds per acre, but this is not satisfactory since the yield averages no more than 600 pounds per acre.

The Use of Potash

We have found many areas in Lancaster County where potash is the limiting factor of growth, and additions of potash have increased yields. In many cases, however, the potash concentration in the soil appears to be sufficient for good yields without further additions, but the tobacco is of inferior quality. Under these conditions, adding potash increases the production of "wrappers" over "fillers", and improves the burning quality more than the addition of any other element.

The form of potash must be considered. While chlorine has been "outlawed" from tobacco fertilizers, nevertheless some muriate of potash is used. Tobacco so treated does not command a good sale. We caution against the use of muriate of potash either to the tobacco crop or to crop preceding tobacco. There is danger in following potatoes or tomatoes with tobacco if muriate of potash has been used in fertilizing these crops.

The Cost

This is of paramount importance. The farmer will use fertilizer only when and if he finds that it is advantageous for him to do so. This is governed to a certain degree by the price he receives for his product. On the other hand, the popularity of Pennsylvania tobacco is evidenced in the form of a good five-cent cigar. Under these conditions, the manufacturers will not pay a price which would force them to charge more for the cigars; in all probability sales would be retarded, affecting not only their own prosperity but also that of the grower. The prices paid for good tobacco, however, should be sufficient and has been sufficient to warrant the farmer using fertilizers intelligently and at a liberal rate.

In conclusion, I see no valid reason why a brand of fertilizer for Pennsylvania tobacco should not have a wide ratio, and contain more phosphorus than nitrogen, and more potash than phosphoric acid. The use of a narrow ratio is not to be recommended.

The Agricultural Future of Puerto Rico

By D. E. Haley

Pennsylvania State College of Agriculture, State College, Pennsylvania

A VISITOR to Puerto Rico soon realizes that he is enjoying a rare privilege. As the ship nears the northern coast, plowing through the deepest waters in the seven seas, the first view of the Island is of rugged mountain ranges, which extend east to west through the interior. These do not exceed 4,400 feet in height, but are quite distinctive and beautiful, with rather sharp peaks and ridges, covered with vegetation. Graceful cocoanut palms, with occasional royal palms, fringe the Island. To one who views tropical vegetation for the first time, the experience is long to be remembered. As seen by Columbus (this was the only "American" soil he visited) it must have been even more beautiful. Some of the native forests still stand. One on the summit of El Yunque Mountain is said to be the most outstanding example of a rain forest in the world. Here giant ferns grow over 20 feet high.

As the steamer approaches the splendid port of San Juan, the largest city on the Island and the point where most visitors land, there appears on the sky line the rugged outlines of Morro Castle. This fortress, which is also beautiful in architectural design, played a prominent part in the early history of Puerto Rico. It was here that Drake, Hawkins, and Cumberland were repulsed when they tried to capture San Juan from the sea. It was here that a shot was fired at a German ship during the World War; this was the only shot fired at an enemy from American soil during that war. Close to Morro Castle is the Governor's Palace, a marvelous structure rich in historical background. It now is occupied by the Hon. Blanton Winship, the Governor of the Island, an able and conscientious administrator, of whom Americans may well be proud.

Not far from the point of disembarkation is the cathedral in which are the bones of Ponce de Leon, who sought to find in the New World the "Fountain of Eternal Youth." Ac-

The Federal Experiment Station at Mayaguez.

cording to some enthusiasts, his mission would have been partially fulfilled if he had discovered Coamo Springs in southern Puerto Rico, the medicinal value of which is internationally recognized.

San Juan is a very interesting city, and is rich in history and traditions. If one expects to find here the modern conveniences of an American city, he is doomed to disappointment. The Spanish rather than the American type of architecture will no doubt prevail for years to come.

Puerto Rico is said to have both a summer and a winter season. To a visitor from the American mainland, however, it seems to have but one continuous summer season. If the Island is visited during the so-called summer season and an auto ride is taken into the interior, the highways are lined with flamboyants which form a beautiful archway over the road. Fragrant, scarlet flowers cover these trees; the petals slowly fall and blanket the road. It is a driveway of "living fire;" the wind, lifting the fallen petals, gives a weird effect of "pulsing flames."

Unrivaled Scenery

At some points, especially on the old Spanish Military Road, one rides dangerously near the edge of a bare precipice. At other points from high elevations there is a view of scenery not duplicated in any other part of the world. One of these high points is Las Mesas, near the city of Mayagüez, where the Federal Experiment Station (headed by Dr. Atherton Lee) and the Agricultural College are located. If this is viewed at the time of a gorgeous Puerto Rican sunset, the grandeur of the scene — mountains, lowlands, and the sea — beggars description. It may be fittingly described only by a poet, an artist, or romanticist—certainly not by a scientist.

The wild life found in similar tropical regions elsewhere is absent here. A stillness permeates the Island. It implies peace. But the Island has not always been peaceful. In the early days, the Caribs, the most warlike of American Indians, were found here. They fought the Boriqueños—people from Boriquen, "Fatherland of Valiant Men," and other Indians, as well as the whites who discovered and colonized Puerto Rico.

Continuous Growing Season

Records at San Juan show a mean temperature of 77.9° F., a maximum of 94° F., and a minimum of 62° F. This is characteristic of the lowlands, but it is cooler in the mountains. Nowhere, however, has frost been known. This temperature assures the Island of a continuous growing season, but some plants thrive better at one time of the year than at another. The sunshine is brighter and more active in Puerto Rico than on the American mainland, since there are less dust particles to obstruct the rays. It is interesting to note that there is an average daily illumination equal to 10.9 hours, notwithstanding the cloudiness of many rainy days.

In the Luquillo Mountains the rainfall averages 145 inches annually. At Ensenada, in the western end of the south coastal plain, it is 26 inches. Here, and in certain other areas, irrigation is practiced. On the average the rainfall is ample over the major part of the Island. Excess rainfall is now turned into an asset, being used for the generation of electric power.

The time is not far distant when illiteracy will be unknown. Even now, most of the laborers are literate. Puerto Ricans are the soul of hospitality. Contrary to what one might expect of those living in a tropical environment, the people are hardworking and ambitious. They are an agricultural people and are willing to undergo hardships in order to obtain a living from the soil.

Puerto Rico is one of the most densely populated areas on the earth,

Above: Tobacco is the second largest export crop of Puerto Rico. This scene taken from a typical farm shows the soil ready for planting. **Below:** Sweet potatoes growing in the foreground, with sugar cane, the largest export crop, furnishing an appropriate background.

Above: A "hacienda" coffee farm near Ponce. Below: Fields of pineapple contribute to the picturesqueness of the landscape.

Above: A tobacco district in the La Plata Valley. Below: Cucumbers growing on the Garcia farm located between San Juan and Cagas.

Above: Experimental work on "terracing" at the Federal Experiment Station at Mayaguez.
Below: An experiment field of Puerto Rican tobacco which received a high potash treatment.

having 501 persons per square mile, one to every 1.42 acres, one to every 0.79 acre of improved land, and one to every 0.49 acre of cultivated land. The population increased 18.7 per cent from 1920 to 1930. The people speak both English and Spanish and uphold the ideals and culture of both nations. They are as familiar with Shakespeare as with Cervantes.

The people love their homeland, and rightly so. While some have located in New York City and certain other parts of the United States, relatively few leave the Island to seek a living elsewhere. With justifiable pride they call their homeland "The Paradise of the Atlantic."

Usually tropical soils of varying degrees of elevation are eroded to a marked extent. This is not true in Puerto Rico. In the tobacco areas of Caguas, Cayey, Comerio, Corozal, Cidra, Ciales, and elsewhere, soils cultivated are on slopes of 45 degrees or more, yet in very few instances does erosion occur. This is due, in large measure, to the nature of the soil and to its comparative freedom from quartz grains.

Variety of Soils

There is a wide variety of soil types due to rough topography, native vegetation and its uneven distribution, the unusual amount of precipitation, and the varying nature of the parent material. In the northern coastal plain the soils are redder, more matured, and more leached of soluble salts than in many other areas.

It would be difficult to find more fertile soils, or soils more suitable for cultivation, than those of the lowlands. These are to a considerable extent alluvial in origin. It is in this locality that the greatest export crop of the Island, sugar cane, is grown. Sugar cane also is grown on soils of slightly higher elevation, where tobacco was formerly grown. This further reduces the acreage which is devoted to tobacco, which is the second largest export crop.

The topography of the soil renders it difficult to sterilize seed-beds properly, although the method now used has proved markedly efficient. Moreover, the soils are rather difficult to cultivate on account of their steepness.

Hurricanes (from Hurican, a Carib Deity) are matters for concern, especially from the standpoint of the destruction of curing sheds; this presents an almost insurmountable difficulty.

Soil Fertility

The greatest problem of the tobacco grower, however, is that of the fertility of the soil. Because of the relatively high temperature and the heavy rainfall, the tobacco soils have been depleted of available nutrients to a considerable extent. This holds true for other soils on the Island as well, with the result that in 1935 there were imported 70,783 tons of fertilizer, valued at $1,434,415. Much of this was used for fertilizing tobacco.

Because of the favorable conditions for nitrogen fixation, these soils contain a considerable quantity of this element. A 6-7-8 fertilizer for tobacco is used in most cases. This appears to have too narrow a ratio for cigar-leaf tobacco; a higher unitage of potash would doubtless prove as beneficial here as it has in other localities where cigar-leaf tobacco is grown.

The Department of Agriculture and Commerce, headed by Commissioner R. Menendez Rámos, who has a well-trained staff, has established a Tobacco Institute, in which the most important phases of the tobacco industry of Puerto Rico are studied. Dr. J. A. B. Nolla, Assistant Commissioner of Agriculture and Commerce, has played a very active and notable rôle in tobacco research in recent years.

At the Insular Experiment Station, studies of Puerto Rican soils are now being made. This work is under the supervision of Director López Domínguez, who has a staff of outstanding scientists, among whom may be mentioned Dr. J. A. Bonnet, who is recognized as an authority on tropical soils.

The value of the research indicated cannot be over-emphasized. Puerto Rico is essentially an agricultural area and has been so since the early days of colonization. While considerable attention has been given in recent years to the processing of agricultural products, to manufacturing, fishing, and the mineral industries; nevertheless, the future of Puerto Rico seems to depend largely on the sugar, tobacco, and other agricultural interests. The rise or fall in the value of agricultural export crops is very closely associated with the well-being of this agricultural people.

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FURTHER STUDIES CONCERNING THE FORMATION OF THE B-VITAMINS IN THE DIGESTIVE TRACT OF THE RAT[1]

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Department of Agricultural and Biological Chemistry, Pennsylvania State College

(Received for publication September 22, 1936)

In previous publications (Guerrant and Dutcher, '34 a, b) it was shown that, under certain experimental conditions, dextrinized cornstarch exhibited unusual dietary properties when compared with sucrose or with the cornstarch from which the dextrinized starch was prepared. The unusual growth responses made by young rats while receiving diets deficient in the vitamin B complex and containing dextrinized cornstarch as the source of carbohydrate have been definitely associated with a characteristic condition existing in the digestive tract of these animals. It has been demonstrated that under such conditions the rat is able to synthesize, in the lower digestive tract, such dietary essentials as are necessary to supplement the vitamin B complex deficient diet. However, in order for the rat to obtain maximum benefits from the products thus synthesized, it is necessary that the animal ingest its excreta, especially the feces.

We have shown (Guerrant, Dutcher and Tomey, '35) that the elaboration of these dietary supplements, by the rat, takes place in the region of the cecum and it was suggested that this elaboration is probably due entirely to the action of certain microorganisms. The marked regularity of the synthesis of these dietary supplements when the rat receives the dextrinized cornstarch diet, and the almost complete lack of such synthesis when comparable diets containing either sucrose or cornstarch are fed, led us to conclude that the above phenomena are due indirectly to differences in rapidity of digestion and subsequent absorption of these carbohydrates. Although it has been frequently observed that, under comparable conditions, greater quantities of dextrinized cornstarch reach the cecum than do either sucrose or unheated starch, much remains to be learned concerning the physiological processes associated with the digestion and the utilization of various forms of carbohydrates. The brief experiments herein reported were carried out with the view of obtaining further information concerning this interesting and important problem.

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EXPERIMENTAL

The investigations of Whipple and Church ('35) have suggested that the marked contrast in the deportment of young rats receiving vitamin B complex deficient diets containing starch, as compared to other rats receiving comparable diets containing sucrose as the source of carbohydrate, might be due to the small quantity of fatty materials held by the starch particles. Such an explanation might appear to be adequate insofar as accounting for the differences in the nutritive properties of dextrinized starch and sucrose, but is wholly inadequate to account for a similar difference in the nutritive behavior of the dextrinized starch diets and the untreated starch diets, the latter being more comparable to sucrose-containing diets in their nutritive behavior than to the dextrinized starch diets. It does appear, however, that an appreciable quantity of fat in the diet might affect the carbohydrate digestion, thus changing the nature of the micro-flora of the lower intestinal tract, thereby affecting the synthesis of the dietary supplements. In fact it would seem that the inclusion of any substance or substances in the diet which would retard the absorption of the carbohydrate from the digestive tract might be a contributing factor.

In order to study the influence of definite quantities of fat on the synthesis of the dietary substances in the digestive tract of the rat, three diets containing sucrose as the source of carbohydrate were used (diets 369, 370 and 371). As will be observed from table 1, these diets were of similar composition and differed only in that diets 370 and 371 contained 10 and 20%, respectively, of hydrogenated cottonseed oil in place of an equal weight of sucrose.

TABLE 1

Giving the percentage composition of the various diets used in the investigation

DIETS	349	356	369	370	371	372	373	374	375	376
Purified casein	18	18	18	18	18	10	10	18	18	18
Salt mixture	4	4	4	4	4	4	4	4	4	4
CellU flour	2	2	2	2	2	2	2	2	2	2
Cod liver oil	2	2	2	2	2	2	2	2	2	2
Dextrin (autoclaved 4 hours)	74	0	0	0	0	0	0	0	0	0
Cornstarch	0	74	0	0	0	0	0	0	0	0
Sucrose	0	0	74	64	54	0	0	0	0	0
Hydrogenated cottonseed oil	0	0	0	10	20	0	0	0	0	0
White bread	0	0	0	0	0	82	0	0	0	0
Brown bread	0	0	0	0	0	0	82	0	0	0
Corn flakes	0	0	0	0	0	0	0	74	0	0
Dextrin (autoclaved 8 hours)	0	0	0	0	0	0	0	0	74	0
Dextrin (autoclaved 12 hours)	0	0	0	0	0	0	0	0	0	74
	100	100	100	100	100	100	100	100	100	100

Since the inclusion of dextrinized starch (starch which had been moistened with 0.1% solution of citric acid, autoclaved for 4 hours under 15 pounds of pressure and dried) in the diet of the rat had been found to result in a very marked increase in the biological value of the feces, it appeared important to know whether further autoclaving of the starch would bring about more marked changes in this direction. To test this out, four equal portions of cornstarch were weighed out, of which three portions were mixed with equal quantities of the dilute citric acid solution in the usual manner and autoclaved under 15 pounds of pressure for 4, 8 and 12 hours, respectively. The three portions of autoclaved starch and a fourth portion of untreated starch were placed simultaneously in a large drying oven where they were kept until rendered free from moisture. When dry, each of the four portions was pulverized and incorporated into comparable diets (diets 356, 349, 375 and 376).

The better nutritional deportment observed among those animals which received the dextrinized starch diets in earlier experiments was apparently due to certain changes in the carbohydrate brought about by its treatment with heat. It, therefore, seemed possible that similar changes in the carbohydrate fraction might be brought about in the baking of bread. If such happened to be the case, a well-browned loaf might be expected to exhibit superior nutritional value. In order to investigate this particular problem, forty fresh loaves of bread, purchased on the local market, were used. Each loaf of bread was separated into two portions; the brown crusty exterior and the white spongy interior. The two bread portions were dried at room temperature, pulverized and incorporated into diets 372 and 373 as sources of carbohydrate. In order to have the protein content of these diets comparable to those previously mentioned, the casein content was reduced from 18 to 10%, as will be observed from table 1.

Continuing the reasoning along the same general line, it would appear that a highly dextrinized product such as corn flakes might exhibit very unusual dietary properties when fed to rats in vitamin B complex deficient diets as a source of carbohydrate. A quantity of this product was, therefore, purchased on the local market, dried, pulverized and incorporated in diet 374 in the proportions given in table 1.

In the nutritional studies each of the above described diets was fed to two comparable groups of young rats (six rats per group), in one group of which the animals had access to their own feces and in the other group the animals were denied access to any excreta. All animals were caged in individual metal cages which were provided with coarse screen bottoms. Fresh water and a liberal quantity of the respective diet were kept before each animal at all times. Body weight changes and food consumption were recorded at weekly intervals. The feces from one group of animals receiving each diet were scraped from the cage pans, placed in a small earthenware container and returned to the cage daily. The feces from the second group of animals were removed from the cage pan daily, the number of pellets counted and then stored in group containers under ether, a different container being used for each group of rats every week. This procedure was followed for 6 weeks for all animals except those which died before the end of this period.

At the end of the experiment, all weekly collections of feces were extracted by shaking successively with four 100 ml. portions of ethyl ether, dried, weighed and their reducing value determined by the A.O.A.C. method ('24) for the determination of starch and other hydrolyzable carbohydrates of feeding stuffs.

Data

Although the experiments were continued for a period of 6 weeks, the mortality among certain groups of animals during the fifth and sixth weeks makes it difficult to compare the responses made by the several groups of animals during the entire experimental period. In order, therefore, to eliminate the factor of mortality from a consideration of the results, only the data obtained during the first 4 weeks of the experimental period are given. These data have been condensed and are given in table 2. The incidence of mortality during the last 2 weeks of the experimental period will be discussed in a subsequent paragraph.

DISCUSSION

It will be observed from the data presented in table 2 that the substitution of 10, or even 20% of hydrogenated cottonseed oil (diets 370 and 371) in the sucrose-containing vitamin B complex deficient diets, for an equal weight of the carbohydrate, resulted in no marked differences in the general deportment of the experimental animals, except a measurable decrease in the amount of diet consumed. This decrease in food intake was to be expected because of the increased caloric value of the fat-containing diets. It will also be observed from these data that those animals which had access to their feces (groups 1, 3 and 5) consumed more of the respective diets and lost less in body weight than did the corresponding groups of animals which did not have access to their feces (groups 2, 4 and 6). These relative differences were not altered by the presence of hydrogenated cottonseed oil in the diet. It does appear, however, that the amount of ether-insoluble fecal matter was increased somewhat as a result of substituting this particular form of fat for an equal weight of sucrose.

TABLE 2

Showing the average deportment of each of the six animals composing the respective groups during the first 28 days of the experimental period

DIET NO.	GROUP NO.	DIETARY SUPPLEMENT	AVERAGE INITIAL WEIGHT	TOTAL CHANGE IN BODY WEIGHT	TOTAL FOOD INTAKE	DRY ETHER-EXTRACTED FECES				
						Total weight	Per cent	Number of fecal pellets	Average weight of fecal pellets	Reducing value of fecal matter
			gm.	*gm.*	*gm.*	*gm.*			*gm.*	*mg.*[1]
369	1	Feces	45	— 3	97	...	...	...	....	...
	2	None	46	—10	84	3.3	3.9	92	0.036	57
370	3	Feces	44	— 2	91	...	...	...	....	...
	4	None	45	—12	78	4.4	5.6	121	0.036	80
371	5	Feces	45	— 4	84	...	...	...	....	...
	6	None	44	—10	69	3.5	5.1	104	0.034	55
356	7	Feces	46	0	82	...	...	...	....	...
	8	None	44	— 7	76	3.3	4.3	103	0.032	43
349	9	Feces	46	22	108	...	...	...	....	...
	10	None	45	— 4	84	7.0	8.3	295	0.024	289
375	11	Feces	46	18	108	...	...	...	....	...
	12	None	46	— 1	90	7.9	8.8	332	0.024	292
376	13	Feces	44	20	107	...	...	...	....	...
	14	None	45	— 3	78	8.4	10.8	353	0.024	327
372	15	Feces	45	94	229	...	...	...	....	...
	16	None	44	90	196	9.4	4.8	287	0.033	230
373	17	Feces	45	73	179	...	...	...	....	...
	18	None	44	78	187	10.0	5.3	269	0.037	280
374	19	Feces	46	11	98	...	...	...	....	...
	20	None	46	0	86	5.8	6.7	220	0.026	138

[1] Reducing values are expressed in milligram equivalents of glucose.

It appears, therefore, from these data that young rats receiving vitamin B complex deficient diets which contain sucrose as the source of carbohydrate are benefited somewhat by having access to their own feces during the first week or 10 days of the experimental period. After this time, such feces appeared to have no supplementing value since the test animals declined in weight in a manner similar to that of the rats which did not have access to the excreta. The presence of 10 or even 20% of hydrogenated cottonseed oil in such diets did not alter the results with respect to rate of growth or maintenance of life. Mortalities among the animals receiving the sucrose diets were restricted to groups 2, 4, 5 and 6 and were five, six, two and four animals, respectively, all of which died during the fifth and sixth weeks of the experimental period.

When commercial cornstarch is moistened with a dilute solution of citric acid, autoclaved for 4 hours under 15 pounds of pressure and subsequently dried, some marked changes are brought about in the dietary properties of this substance. This fact may be brought out by comparing the results obtained from animals receiving diets 356 and 349. The general responses of those animals which received the diet containing the untreated starch (diet 356) were comparable to the responses made by animals receiving the sucrose diets, while those animals which received the dextrinized starch diet (diet 349) exhibited a much more favorable nutritional picture. As has been previously reported, those animals which received the dextrinized starch diet and which also had access to their own feces grew at a very favorable rate (22 gm. in 4 weeks), while those animals which received a similar diet, but did not have access to the feces, not only failed to grow but actually lost weight. This loss in weight was not so great, however, as had resulted among comparable groups of animals which had received the starch or the sucrose diets.

Other noticeable changes, in addition to the increase in growth rate, resulted from the dextrinization of the starch. The percentage of ether-insoluble fecal matter was almost doubled, the number of fecal particles was trebled, the average weight of the fecal particles was reduced approximately one-fourth and the reducing equivalent of the fecal matter was increased sevenfold.

These data indicate quite clearly that dextrinization of cornstarch according to the method described, renders it less readily digested by the normal digestive processes of the rat. Such findings could not have been predicted from our present conception of carbohydrate hydrolysis and metabolism. The complete explanation for the lowered digestibility of the dextrinized cornstarch will have to await further studies. It appears, however, that during the process of dextrinization and subsequent drying, a portion of the carbohydrate fraction is, in reality, dehydrated or caramelized. When ingested by the rat, the caramelized carbohydrate must first become hydrated before it can be acted upon by the hydrolytic agents. This delay in digestion, along with the mild laxative effect of the caramelized products, tends to increase the amount of nutrients reaching the lower bowel and, in consequence, greatly encourages the growth of yeast or similar microorganisms. This appears to be the most probable explanation for the syntheses of the B-vitamins in the digestive tract of rats receiving dextrinized cornstarch diets.

Further autoclaving of the starch for 8 and 12 hours, respectively, did not greatly alter the nutritional properties of the starch beyond that which had been obtained by the 4-hour autoclaving (diets 375 and 376). There did appear to be some increase, however, in the per cent of fecal matter, in the number of fecal pellets and in the amounts of reducing substances in the feces, as a result of the longer periods of autoclaving. These increases were not great. The mortalities among the animals receiving the starch diet or the dextrinized starch diets were restricted to groups 8, 10, 12 and 14 and were three, two, one and one rats, respectively, all of which died during the sixth week of the experiment.

The growth responses obtained as a result of feeding groups of young rats diets containing different parts of the baker's loaf (diets 372 and 373) were not according to expectation. It is highly probable, however, that these results were greatly influenced by the presence of considerable amounts of the B-vitamins in the original bread. All animals receiving the diets containing the bread made very unusual growth responses. In fact, the growth rate was so rapid that any beneficial effect of feces to those animals was obscured. The somewhat suppressed growth rate of those animals (groups 17 and 18) which received the diet containing the outer portion of the loaf (diet 373) indicates that the growth-stimulating value of the loaf had been reduced by the baking process. In all probability this reduced growth rate was due to a partial destruction of the B-vitamins near the exterior of the loaf.

The dietary supplementary value of the feces from those animals which received the diet containing corn flakes (diet 374) was quite definite, but not so marked as had been observed among those animals which had received the diets containing the dextrinized cornstarch diets. From the data obtained from the animals receiving this diet, as well as from those animals receiving the dextrinized starch diets (as contrasting those animals which had received the sucrose and the starch diets), it appears that a relatively large percentage of fecal matter of low density and of high reducing value is associated with the formation of substances in the digestive tract of the rat, which are effective in supplementing a vitamin B complex deficient diet. A large fecal output, composed of numerous pellets of relatively high reducing value, is believed to be indicative of a definite laxative effect in the digestive tract, while few fecal particles of low reducing value might indicate the opposite condition. In the above experiments, however, the average weight of the individual fecal particle bore little if any relationship to their average size. The fecal pellets from the dextrin-fed rats were invariably large, fluffy and highly aerated, while those from the sucrose-fed rats were somewhat smaller in size and were very compact. The superior physical condition of the digestive tract of the dextrin-fed rats, as contrasting the sucrose-fed rats, was always apparent.

The recent contribution of Bliss and Green ('36) has greatly extended our knowledge concerning the phenomenon of 'refection.' While it is generally considered that refection and those conditions which cause coprophagy to vitiate experimental results are different phenomena, there remains the possibility of some relationship existing between the two conditions. In fact, several factors point toward the existence of such a relationship. While it is entirely possible that the two phenomena are caused by different types of microorganisms, it is also possible that both are caused by the same type of organism but in different parts of the rat's digestive tract. It seems reasonable to suppose that in the case of refection, where there is a direct absorption of the growth-stimulating factors, the elaboration must take place in the upper digestive tract, probably in the small intestines. Since in the other case, it is necessary that the rat ingest the excreta in order to receive full benefit of the products formed, this suggests that the elaboration takes place in the lower gut. The fact remains that in one case the supplementary substances are absorbed directly from the digestive tract, while in the other it is necessary that the animal re-ingest the feces in order to receive full benefit from the products synthesized in the rat.

SUMMARY

Further studies have been made concerning the role of the diet in the formation of the B-vitamins in the digestive tract of the rat. From results obtained during this investigation, the following conclusions are drawn:

1. The presence of 10 or 20% of hydrogenated cottonseed oil in the vitamin B complex deficient diet, containing sucrose as a source of carbohydrate, does not lead to a more favorable production of the B-vitamins in the digestive tract of the rat.
2. Further autoclaving of the starch beyond the usual 4-hour period does not lead to a significant increase in the amount of supplementary substances eliminated by the rat in its feces.
3. Those diets which, when fed to rats, result in relatively large percentages of fecal matter, having a low density and a high reducing equivalent, are conducive to the formation of substances in the digestive tract which are effective in supplementing a vitamin B complex deficient diet.

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CHEMISTRY OF FOOD AND NUTRITION. *Henry C. Sherman,* Mitchill Professor of Chemistry, Columbia University. Fifth edition. The Macmillan Co., New York City, 1937. x + 614 pp. 38 figs. 13 × 20 cm. $3.00

The purpose of this book is "to present the principles of the chemistry of food and nutrition as an integral part of the study of chemistry and with reference to the food requirements of man, and the considerations which should underlie our judgment of the nutritive values of foods and the choice and use of food for the advancement of positive health."

The favorable reception which former editions have received and the fact that a fifth edition has become necessary are ample proofs that the author has succeeded in accomplishing his purpose.

The general plan which characterized the fourth edition has been adhered to in the present edition, with the exception that the number of chapters has been increased from twenty-four to twenty-seven. Chapter IX of the former edition, entitled "Conditions Governing Energy Metabolism and Total Food Requirements," has been expanded into two chapters in the new edition which are entitled, "The Basal Energy Metabolism Regulation of Temperature, and Specific Dynamic Action" and "Total Energy Metabolism and Food Requirement."

Two new chapters have been added, *viz.*, "Food Economics in the Light of the Newer Knowledge of Nutrition" and "Food Chemistry and Human Progress." In the latter chapters the author discusses, in a practical way, the important problem of correlating food prices with food values and the importance of proper nutrition in relation to longevity.

In general, the entire book has been rewritten and brought up to date. The author has the remarkable ability of choosing the most important and significant material, and of condensing a mass of valuable information into a relatively small space, without doing so at the expense of clarity or scientific accuracy.

The reviewer can do no more than repeat what he wrote in reviewing the fourth edition of this excellent book, *viz.*, "Dr. Sherman's new text will commend itself to every teacher and student who desires a well-printed text containing a wealth of chemical and nutritional information interpreted by an outstanding teacher and research worker."

R. ADAMS DUTCHER

THE PENNSYLVAIA STATE COLLEGE
STATE COLLEGE, PENNSYLVANIA

COUNTY SOIL A

Local Farmers Have Kept Land

Dr. D. E. Haley, professor of Soils and Phytochemistry at the Pennsylvania State College, has prepared a special article for publication in the Lancaster Newspapers for the benefit of Lancaster county tobacco growers. It is entitled "Farm Manure as a Fertilizer for Cigar-Leaf Tobacco" and will be issued in pamphlet form by the college.

"I wish that evey tobacco farmer would read this article, as it has a direct bearing on questions relating to the yield and quality of cigar-leaf tobacco," Dr. Haley stated in submitting the article.

He points out the "striking results" in quality and yield which were obtained by Otto Olson in five years' work at the local Tobacco Experiment Station.

Dr. Haley's article follows in full:

The utilization of farm manures for the maintenance and improvement of the fertility of agricultural soils has been a common practice for many centuries. Through the ages the efficiency and thrift of a farmer, in many instances, has been gauged by his interest in the care and use of this material.

There is no valid reason why farm manures should not be used as soil additions, since in this way at least a partial restoration is made of mineral nutrients taken from the soil by crops, which otherwise would represent a permanent loss of plant food. Many questions are involved, however, as to how manure may be used to best advantage from the standpoint of yield and quality of crop, and especially cigar-leaf tobacco.

For many generations Lancaster County farmers have fed a large portion of their crops to farm animals, especially to steers, utilizing the manure to maintain the fertility of their soils. The present excellent productivity of these soils, which have been under cultivation for more than two centuries, has been attributed by some as due mainly to this practice.

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COUNTY SOIL AIDED BY USE OF MANURE

Local Farmers Have Kept Land Productive By Proper Application of Fertilizer

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The utilization of farm manures for the maintenance and improvement of the fertility of agricultural soils has been a common practice for many centuries. Through the ages the efficiency and thrift of a farmer, in many instances, has been gauged by his interest in the care and use of this material.

There is no valid reason why farm manures should not be used as soil additions, since in this way at least a partial restoration is made of mineral nutrients taken from the soil by crops, which otherwise would represent a permanent loss of plant food. Many questions are involved, however, as to how manure may be used to best advantage from the standpoint of yield and quality of crop, and especially cigar-leaf tobacco.

For many generations Lancaster County farmers have fed a large portion of their crops to farm animals, especially to steers, utilizing the manure to maintain the fertility of their soils. The present excellent productivity of these soils, which have been under cultivation for more than two centuries, has been attributed by some as due mainly to this practice. No doubt it has been an important factor.

A certain proportion of the manure produced in the tobacco area of Lancaster County is hauled to the field immediately before planting corn. This practice has a great deal of merit, since the corn plant is a coarse feeder and the deleterious effects of such an application, if any, are reduced to a minimum. What is true for corn, however, may not be true for wheat and for other crops, such as cigar-leaf tobacco.

While no trustworthy figures are available. it is believed that the largest proportion of the manure produced in the Lancaster County tobacco area is applied immediately preceding the growing of tobacco. In many instances, unfortunately, this is the sole fertilization for tobacco. In some cases the manurial treatment is reenforced by additions of commercial fertilizers. In isolated cases, manure is not used at all or is applied one year previous to the planting of tobacco.

THE COMPOSITION OF MANURE

The composition of farm manure varies to a considerable degree. It is affected by the age and kind of animal, the utility of the animal, the kind and quanity of feed consumed, the nature of the bedding material and the care of the manure produced and other factors. Experiments at The Pennsylvania State College indicate that fresh manure with bedding, as it comes from the cow barns, contains an average of 12 pounds of nitrogen, 5 pounds of phosphoric acid and 9 pounds potash per ton. Usually the figures are quoted as 10, 5 and 10 respectively. In no case, however, according to the Ohio Experiment Station, is more than 80 per cent of the amount of these plant foods that were present in the materials when fed, ever returned to the soil in the form of manure. With the most rigid precautions, considerable quantities of the fertilizing constituents are lost through leaching and evolution of gases, in addition to the amount used by the animal.

Most of the phosphoric acid in manure is in the solid portion. Usually the nitrogen is equally distributed between the solid and liquid portions. At least 75 per cent of the potash is in the liquid portion. These figures clearly indicate that manure is likely to lose considerable of its fertilizing value, especially nitrogen and potash, if subjected to leaching during storage.

MANURIAL CONSTITUENTS

Most of the nitrogen of manure is readily available for plant growth. The phosphoric acid is rendered available only through decomposition. The potash, if present, is quite available, there being no insoluble compounds of this element present in manure.

Manure contains a large quantity of carbon, in the form of straw and similar materials, especially when it is fresh. This is a source of energy for the growth of organisms in the manure, and to other organisms after it is incorporated with the soil. In order that these may grow and multiply, they require, among other things, a considerable quantity of nitrogen and phosphoric acid, and a lesser quantity of potash. They appear to have little difficulty in competing with the tobacco plant for these foods. When fresh manure is applied alone to tobacco, these organisms may seriously compete with the tobacco for plant food constituents and may interfere with the early development of the plants. With the death of these organisms, later in the growing season, the plant food elements that they have used may become available through decomposition.

MANURIAL APPLICATIONS

The application of fresh manure to a soil invariably leads to a depletion of its available nitrogen for the time being. For this reason, if for no other, manure should be applied some time previous to the growing of tobacco so that this effect may be reduced to a minimum by allowing some decomposition to take place. There is less danger if the manure is well rotted before application, providing there is little loss of fertilizing constituents during this period.

The application of fresh manure is far more harmful during dry than wet years, because the tendency is toward a loss of water from the soil through the heating of decomposition. Undue aeration may likewise be induced. This effect on plants is usually referred to as "burning". It is not evident when well rotted rather than fresh manure is used.

As a rule, the application of manure improves the physical, chemical and biological conditions of the soil to a certain degree. As a source of organic matter, its value has probably been over-estimated, but as not less than 10 tons per acre are used some humus formation will occur. This results in a better moisture holding capacity of the soil and a better retention of many mineral nutrients against the forces of leaching, conditions which materially benefit plant growth.

Some investigators maintain that one of the beneficial effects of manure is that there are liberated on decomposition certain compounds which stimulate plant growth, similar to the effects of certain vitamins on animal growth. Be that as it may, there is no reason why the same effect should not be noted with cottonseed meal or green manuring, if the same microorganisms are present.

MANURE AS TOBACCO FERTILIZER

While all authorities do not agree as to the merits or demerits of applications of manure for the tobacco crop, they do agree that manure is, at best, an unbalanced fertilizer, especially for tobacco. It is usually looked upon as a source of nitrogen rather than as a source of phosphoric acid or potash. It may, because of its unbalanced condition, induce the development of plants of low resistance to certain diseases. Experiments at The Pennsylvania State College and elsewhere have indicated the desirability of reenforcing manure with phosphoric acid; crop yields are materially increased as compared with manure used alone, and more normal development is obtained. In the case of cigar-leaf tobacco, manure should be reenforced by both phosphoric acid and potash, since high quality as well as high yields is desired. The early work of Dr. William Frear on Pennsylvania tobacco showed this. He found that the increase in yield was usually more than sufficient to pay for the cost of the fertilizer used and that the "burn" was decidedly improved.

In recent years, striking results were obtained in five years' work reported by Otto Olson in Pennsylvania Experiment Station Bulletin No. 179. When no fertilizer was used the yield averaged 1,230 pounds per acre. With 10 tons of manure per acre the yield was 1770 pounds. When the manure was reenforced with 300 pounds acid phosphate and 200 pounds of sulfate of potash, the yield was 1,985 pounds. When reenforced with 500 pounds of cottonseed meal, 300 pounds acid phosphate and 224 pounds sulfate of potash, the yield was 2,190 pounds. It was stated, that these striking results in yield were exceeded by the improvement in quality and burn.

If manure is to be used for Pennsylvania cigar-leaf tobacco, care should be taken to prevent the loss of nutrient materials during the storage period. If it is not convenient to allow it to become well rotted. it should be applied at least two months previous to the growing of the crop. Notwithstanding the fact that manure is looked upon as a nitrogen addition, it should be reenforced with a small quantity of readily available mineral nitrogen, as for example, nitrate of potash, to off-set the ill effects which might otherwise be experienced in the early stages of growth. It should be reenforced with both phosphoric acid and potash in order to meet the demands of this particular crop. In no case should it be looked upon as a complete fertilizer for cigarleaf tobacco.

Reprinted from the Intelligencer Journal, Lancaster, Pa.,
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BULLETIN 344 APRIL, 1937

Deposition and Retention of Sprays on Apples

APPLYING A COVER SPRAY IN THE EXPERIMENT ORCHARD AT STATE COLLEGE

The Pennsylvania State College
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
State College, Pennsylvania

DIGEST

The deposition and retention of lead and arsenic trioxide were determined by analyzing samples taken before and after each cover spray application, and at harvest.

In 1934, 17-year-old Stayman and York Imperial trees were sprayed 6 times, the applications averaging 12 gallons per tree at 400 pounds pressure. The 3 spray mixtures contained lead arsenate at 3 pounds per hundred gallons. Mixtures A and B also contained 2 gallons of liquid lime sulphur, mixture B 2 pounds of skimmilk powder, and mixture C 5 pounds of dry flotation sulphur and 1 quart of menhaden fish oil. Samples of foliage and fruits were taken from the lower branches.

Spray deposits were greater on Stayman than on York Imperial, but losses between spray applications were greater from Stayman.

The weathering away of spray deposits was a negligible factor compared with losses per unit area of fruit surface due to growth.

The foliage was practically full grown by the second cover spray, but the fruits had reached only about 7 per cent of their final area at the third cover spray, and 12 per cent at the sixth cover spray.

On the foliage, mixture C gave the greatest spray deposits and the smallest losses between spraying dates. After the sixth cover spray, leaves in this treatment showed 470 milligrams of lead and 280 milligrams of arsenic trioxide per square meter, or increases of 4 and 5 times respectively over the amounts found after the first cover spray.

On the fruits, mixture A gave the greatest deposits of lead and arsenic trioxide, but mixture C gave the smallest losses between spraying dates. After the sixth cover spray the greatest deposits were 350 milligrams of lead and 140 milligrams of arsenic trioxide per square meter, increases of less than 1.5 times the amounts present after the first cover spray.

Subsequent to the last cover spray residues were retained best from mixture C. By harvest, lead residues on the fruits were from 1/6 to 1/12 of those on the foliage, and arsenic residues 1/10 to 1/16, due to expansion of the fruit surfaces. The fruits retained lead in greater proportion than the foliage.

In 1935, mixture C was applied to Stayman in three different cover spray schedules, and fruit samples were taken from both upper and lower branches.

Top fruits grew more rapidly and showed larger losses of spray deposit per unit area than fruits on the lower branches.

Uniform spacing of spray applications at 14-day intervals, and with intervening 7-day sprays to the tops alone, showed either that early season coverage was poor, particularly in the tops, or that spray deposits became excessively heavy in the later sprays.

The experimental evidence suggests the need for testing, under conditions of heavy codling moth attack, a schedule of 5 cover sprays spaced at intervals of 5, 9, 13, and 17 days, the last application to be made to the tops only. Recommendations must await the results of this test.

Deposition and Retention of Sprays on Apples[1]

DONALD E. H. FREAR AND H. N. WORTHLEY

THE ANNOUNCEMENT of legal tolerances for arsenic and lead on food products has stimulated a search for insecticides that will equal or exceed lead arsenate in effectiveness, but that will not leave poisonous residues on the marketed products. In some directions, notably in the control of truck crop insects, the search has been attended with considerable success. In the control of codling moth (*Carpocapsa pomonella* L.) on the apple, however, it has become evident that lead arsenate possesses an effectiveness not as yet equalled by available substitute materials, and that extended investigation will be necessary before a substitute satisfactory in all respects will replace this standard insecticide.

Accompanying the search for substitutes for lead arsenate is an effort to find means of improving the performance of lead arsenate in the first brood codling moth sprays, and thus lighten the problem of controlling the second and later broods. This line of attack offers considerable promise in Pennsylvania, where there is but one full brood of larvae, a partial second brood, and no known third brood. Under these conditions a high degree of control of the first brood of larvae, which attacks the apples from late May until early July, will leave such small numbers of the pest that a dangerous second brood cannot be produced, and spray applications in late July and August will be unnecessary.

To be effective, an internal poison like lead arsenate must be placed where the newly hatched larvae cannot avoid it at the time of attack; and it must be present in such form and amount that the highest possible percentage of the larvae will get a killing dose while they are seeking to penetrate the apple surface.

Purpose of the Experiments

The experiments of 1934 were designed to answer the following questions:

1. Using lead arsenate and lime sulphur at standard dosage in a standard spray schedule, what amounts of arsenic and of lead are actually deposited on the foliage and fruits of apple?
2. What influences do growth and weathering exert on the retention of spray deposits by these surfaces?
3. Does the variety of apple have any effect upon the deposition and retention of lead and arsenic?
4. Do lead and arsenic disappear from the sprayed surfaces at the same rate?
5. Can any materials be added to spray mixtures of lead arsenate and sulphur fungicides to raise the initial deposit or to aid in retention without complicating the problem of removal?

The experiments of 1934 revealed a spray mixture that is more adhesive than the "standard" lead arsenate and lime sulphur. They showed that growth of the young apples is a far more important influence than weathering on the disappearance of spray deposits. These findings were the basis of the experiments of 1935, which sought to improve the uniformity of the lead and arsenic deposits through changes in the time and manner of application of the most adhesive spray mixture.

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Factors Affecting the Deposition and Retention of Sprays

A study of the factors affecting the amount of deposit on plant surfaces may be divided into two parts: first, an investigation of those processes which result in the deposition upon the plant surfaces of the materials which may be present in solution or suspension in the spray mixture; and second, those processes which occur subsequent to this depositing process, and which, over a period of time, determine the amount of the deposited materials which remain upon the surfaces. For convenience, the former process is designated in this bulletin as the deposition, the latter as the retention of the spray materials. The length of time over which each of the two processes operate is considerably different. Deposition is completed in a few minutes from the time the spray ingredients are applied. The processes affecting the retention are operative over several months. Deposition and retention of sprays, then, while related through factors that affect both, will be discussed separately.

Deposition—The factors which, in the main, determine the deposition of a spray on a plant surface are the chemical and physical properties of the spray mixture and the character of the surface itself. Other variables undoubtedly play a part, but the influence of temperature, humidity, wind velocity, and others are of secondary importance in most cases.

The factors of major importance in determining the deposition of a spray are those which influence the wetting and spreading of liquids on solids. Cooper and Nuttall (3), Hamilton (8), Moore (11) and Woodman (19) have discussed these factors. Certain phases of wetting and spreading phenomena are pertinent to this problem, and will be considered here.

When a drop of liquid is placed upon a solid surface which it does not wet, the angle of contact is 180°. No wetting has been done in this case. This situation obtains when a liquid of high surface tension, such as mercury, rests on the waxy surface of an apple. When the drop of liquid comes in actual contact with a surface which it really wets, however, the course of subsequent events depends broadly on the magnitude of three forces—the surface tension of the liquid, the apparent surface energy of the solid, and the interfacial tension between liquid and solid. Under actual wetting, the surface energy of the solid will tend to pull the liquid out into a film with the maximum surface. Opposing this force will be the surface tension of the liquid and the interfacial tension. When the three forces are in equilibrium the liquid will cease to spread upon the solid.

Any influence, then, which causes a change in the surface tension of the liquid, or in the surface energy of the solid, or in the interfacial tension, directly or indirectly, will affect the spreading of the drop. The ability to wet a surface apparently is a function of the surface tension to a certain extent, although Moore (11) speaks of the phenomenon of wetting as a "slight chemical affinity exhibited between liquid and solid." The terms "wetting agents," "spreading agents," and "adhesive agents," which commonly are used as synonyms in discussions of sprays, are not so in reality. Wetting and spreading abilities are properties which are mutually dependent to a certain extent, for true spreading cannot take place until wetting occurs. Adhesiveness, however, involves a further set of physical properties; these will be considered later.

The materials which are most commonly used as wetting agents and spreaders are the soaps, certain proteins, particularly casein in the form of calcium caseinate, and the sulfonated higher alcohols, fatty acids, and hydrocarbons. These materials produce a more or less continuous film, covering the entire surface to which the spray mixture is applied. Too large an excess of materials with a high surface tension activity will cause a loss of spray materials from the surface, however, through run-off resulting from the action of gravity.

The second class of materials used in spray mixtures to increase deposition are the adhesives, usually some form of oil. These are not, in general, capable of lowering surface tension to any considerable extent, but depend on their adhesive nature. On the surface of the apple fruit, which is coated with a waxy layer, and to a lesser extent on the leaf surface, these oils tend to form an intimate union with the coatings and thus adhere very strongly. Whether this union is physical or chemical has not been determined. The fact that oily substances are often mutually soluble but immiscible with water would indicate that solubility plays a part in their behavior. Since they are applied in the form of emulsions, these materials possibly increase the amount of spray material deposited by a process of mechanically enmeshing the suspended particles. Whatever the cause, they have been shown to be efficient adhesives by Hood (9) and Porter and Sazama (13).

Retention—The economic importance of producing a spray deposit which will not weather away rapidly under the influence of rain and dew is obvious. Smaller quantities of toxic materials may be used, and applications may be made less frequently if the amount lost from the plant surfaces is small. This is particularly important in a crop which requires several months to mature, and is subject to insect attacks over the greater part of that period, as the apple.

Under practical conditions, the external factors which affect the retention of spray deposits are not subject to control. The amount and intensity of the rainfall is variable, as is the temperature, also. Internal factors, those operating directly upon the plant surface and tending to remove the spray deposit, depend on the constituents and concentration of the spray deposit and on the type of plant surface.

Those materials which form a chemical union with the plant surface may be expected to adhere most strongly and consequently to resist the processes of weathering best. Those which adhere only through physical forces should be considerably easier to remove. Probably the oils ultimately form mutual solutions with the waxy surfaces of the plant where such wax exists. It has been suggested that since certain of the constituents of the spray mixture, specifically lead, are basic in character, they may form salts with some of the organic acids present in apple wax (Cohee and St. John (2)). If the oils used were of animal or vegetable origin, it also is quite probable that oxidation of the oil film may produce an impervious surface similar to a varnish. Even if such were not the case the oil undoubtedly furnishes a matrix which is resistant to water and in which particles of the solid material of the spray mixture may be embedded.

The adherence of the other constituents of the spray mixture probably is due quite largely to physical forces. The leaf surface being, in the main, negatively charged, exerts some attraction for those particles which have the opposite sign. The small size of the particles present increases the surface area tremendously, and attractive forces between the particles themselves and between them and the plant surfaces may be considerable. The purely mechanical action of the wedging of some of the solid particles in interstices in the plant surface may be a factor of some importance.

Against these forces tending to hold deposited material to the plant surface are forces which tend to dislodge it. The expansion of the plant surface due to growth exerts a powerful mechanical action on the deposit. The solvent action of dew and rain, particularly upon particles of material exhibiting such large surfaces in relation to their mass, is considerable. The hydrolytic action of such moisture upon the chemical compounds would have the effect of breaking down the more complex units into simpler, and usually more soluble, compounds. Probably such is the situation in the case of lead arsenate, which may be hydrolyzed into lead hydroxide and an acid of arsenic which is more soluble than the original material. The effect of dissolved CO_2 in the water on the plant surfaces is undoubtedly of considerable importance, also.

With such an array of complex and variable factors to deal with, their individual evaluation becomes an almost endless task. The behavior of certain spray mixtures under natural conditions of application has been studied. Hamilton (7) investigated the deposition and rate of loss of arsenic trioxide on Baldwin and Grimes Golden foliage and fruit during the season of 1927 under New Jersey conditions. He found a direct relation between increase in leaf size and decrease in arsenical residue on the leaves. After leaf growth ceased, late in June, there was a more gradual loss, due to weathering alone. On apples, the decrease in arsenical residue was also found to be proportional to the increase in surface. Spuler and Dean (14) conducted similar studies on arsenical residues and emphasized that the loss of arsenic trioxide per unit area of the fruits may be as great as 3.6 per cent daily between cover sprays, due mostly to an increase in size of the apples. Webster (17) and Webster and Marshall (18), reporting work done in 1933, state that the higher the deposit of arsenic, the more efficient the codling moth control, other factors being equal. They studied the relative deposits produced by various combinations of spray materials, and pointed out the increase in retention of the residue obtained by the addition of mineral or fish oils. Different amounts of residues were retained by different varieties, greatest retention being on Winesap followed, in order, by Jonathan, Delicious, and Rome.

These studies throw considerable light on the factors which influence deposition and retention of lead arsenate on apple surfaces, but they are concerned only with the levels of arsenic trioxide occurring at various times during the season. The deposits of lead are not considered, and the two aspects of the problem, deposition and retention, are not considered separately. Obviously, the amount of a spray ingredient discovered on a plant surface at any time will be a reflection of the total effect of all factors concerned. Whether the amount found is the result of high initial deposits or of efficient retention can be determined only by a series of analyses so spaced in time as to allow these factors to be evaluated separately.

The Amount of Lead and Arsenic Present throughout the Season on the Fruits and Foliage of Apples

The work in 1934 was planned to indicate the relative deposit and retention of lead and arsenic on the fruits and foliage of York Imperial and Stayman apples when sprayed with lead arsenate mixtures containing modifying materials, as compared to a standard mixture of this material combined only with liquid lime sulfur. The modifying materials used were menhaden fish oil and skimmilk powder. These were chosen because they are typical of the two classes of materials offered for use in apple sprays; fish oil is a widely used adhesive, and skimmilk powder represents a class of spreading and wetting agents in common use.

The removal of the residues produced by these spray treatments has been reported (6).

Plan and Conditions of the Experiments—This work was done in the experimental orchard at the Pennsylvania State College during the season of 1934. Preliminary studies were made during the season of 1929, but consecutive samples were not taken. While, in general, the results were the same in both seasons, only the 1934 results are presented here.

Thirty-six trees, 18 each of Stayman and York Imperial varieties, were used. They were 17 years old in 1934 and were in moderate to good vigor.

The season of 1934 exhibited nearly normal climatic conditions. Table 1 shows the mean temperatures and precipitation for the six summer months at State College and the normal values for these months. The temperatures during May and June were slightly above normal. The rainfall during May and October was below normal, but during September it was twice the normal. The normal figures in Table 1 are the 50-year averages at the Experiment Station. Figure 1 shows the distribution of the rainfall during the season of 1934.

TABLE 1. MONTHLY MEAN TEMPERATURES AND INCHES OF RAINFALL DURING 1934 GROWING SEASON AT STATE COLLEGE, PA., COMPARED WITH NORMAL

	Mean temperature		Rainfall, Inches	
	1934	Normal	1934	Normal
May	62.4	58.78	1.46	4.12
June	73.9	66.39	4.14	4.27
July	74.6	76.20	3.37	3.84
August	65.3	68.49	3.35	3.57
September	64.4	62.65	6.17	2.98
October	50.8	51.07	1.27	2.64
Total			19.76	21.42
Average	65.2	63.00		

Spray Treatments—Three spray mixtures were applied to each of the two varieties studied. Treatment A was lead arsenate 3 pounds and lime sulfur 2 gallons per 100 gallons of spray mixture. Treatment B was lead arsenate 3 pounds, lime sulfur 2 gallons, and skimmilk powder 2 pounds per 100 gallons of spray mixture. Treatment C was lead arsenate 3 pounds, flotation sulfur 5 pounds, and fish oil 1 quart per 100 gallons of spray mixture. Flotation sulfur was used because of the incompatability of lime sulfur and fish oil. The lead arsenate used in all sprays was the same and contained no deflocculator.

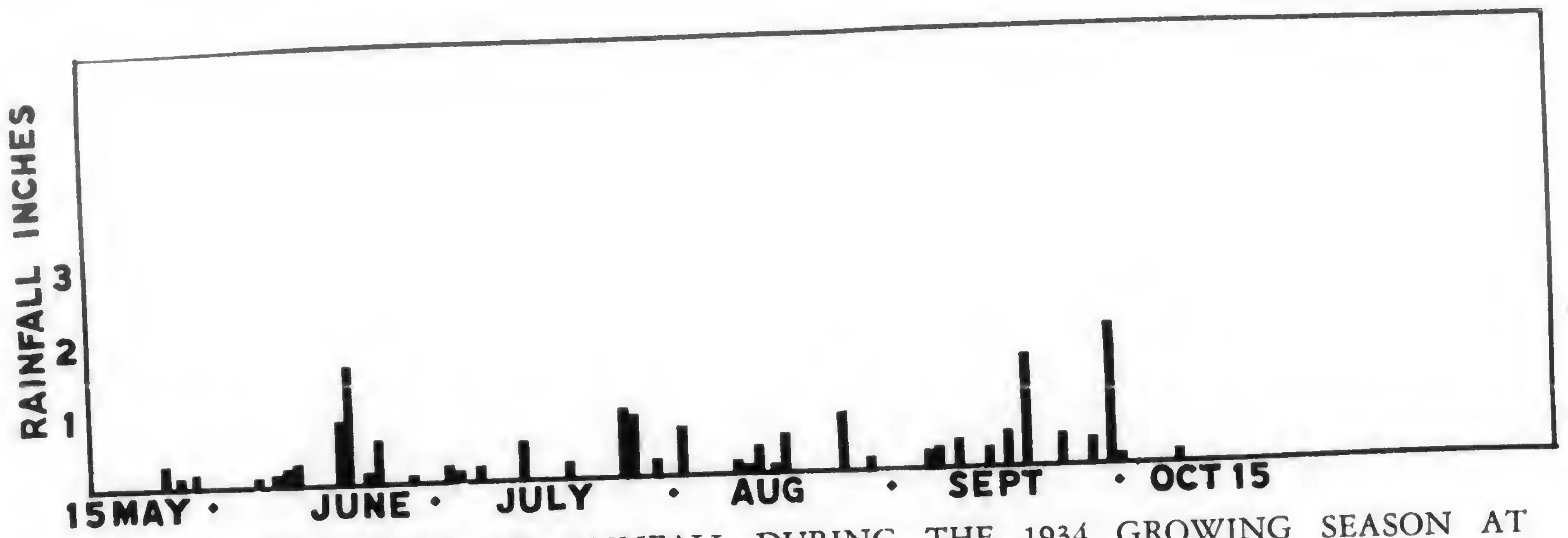

FIG. 1. DISTRIBUTION OF RAINFALL DURING THE 1934 GROWING SEASON AT STATE COLLEGE, PA.

The trees received uniform spray treatment through the petal fall application; differential treatments were confined to the cover sprays. The first cover spray was applied on May 28, the second on June 7, the third on June 16. For the fourth, fifth, and sixth cover sprays, applications were made to only 18 trees on June 26, July 6, and July 16. Samples of fruit and foliage were taken from all trees before and after each application. The weather on all spraying dates was clear, with the exception of June 16 and June 26, which were partly cloudy.

An attempt was made to attain uniformity in spray coverage. The applications averaged 12 gallons per tree. They were applied from the ground with a single nozzle spray gun operated at 400 pounds pressure.

Foliage and Fruit Samples— Each foliage sample consisted of 100 leaves picked from the lower limbs, mainly between the third and sixth leaves on the terminal growth. The leaves were taken to the laboratory immediately, weighed while fresh, and measured with a photoelectric apparatus (5) to determine their area. They then were dried in a steam heated oven at approximately 80°C. When thoroughly dry, they were weighed, ground, and stored in glass-stoppered bottles. A 1-gram sample was digested with HNO_3 and H_2SO_4 in a Kjeldahl flask, and finally made up to a volume of 500 ml. Portions of this solution were taken for analysis for lead and arsenic.

Each fruit sample, with the exception of the final sample, consisted of 20 apples picked from the lower limbs. They were handled as little as possible and were carried to the laboratory in specially constructed boxes, each apple being impaled through the calyx on a pointed nail to avoid removing the spray residue by contact with other fruit or the sides of the container. At the last sampling date, 700 grams of fruit were taken for analysis. Fresh weights were taken immediately. The spray residue was removed by washing in a boiling solution containing 1.5 per cent HCl and 1.0 per cent NaCl by weight.

After all the fruit in each sample had been washed, the washings and rinsings were made up to a volume of 500 ml. and stored for future analysis. The volume of water displaced by each sample of apples was determined by immersing the washed fruit in water in graduated cylinders. Aliquot portions of the digested leaf samples and of the apple washings were analyzed for arsenic by the official Gutzeit method (1), and for lead by the method of Frear and Haley (4).

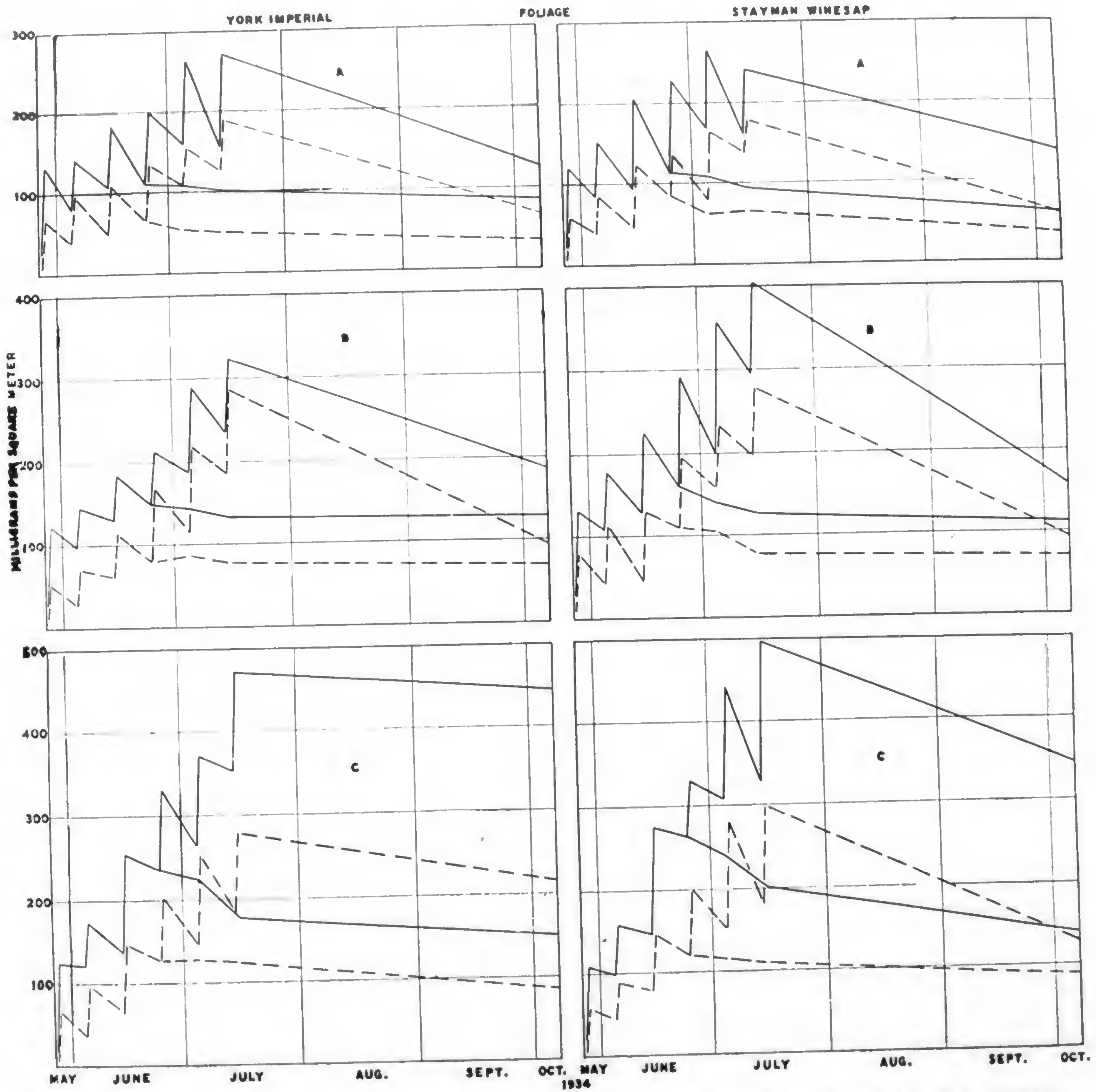

FIG. 2. LEAD AND ARSENIC (AS As_2O_3) ON FOLIAGE BEFORE AND AFTER SPRAY APPLICATIONS AND AT HARVEST

The solid lines represent lead, the dotted lines arsenic. The course of the deposit on the foliage of trees receiving three sprays also is shown.

Experimental Results

Lead and Arsenic on the Foliage—Figure 2 shows the amounts of lead and arsenic found on the foliage samples taken during the season, expressed as milligrams of lead and arsenic trioxide per square meter of leaf surface.

TABLE 2. AVERAGE DEPOSIT OF LEAD AND ARSENIC ON FOLIAGE IN SIX COVER SPRAYS, AND AVERAGE LOSS OF THESE ELEMENTS BETWEEN SAMPLING DATES

Treatment	Deposit Amounts Present		Average deposit	Relative deposit	Loss Amounts Present		Average loss	Relative loss
	Before spraying	After spraying			After spraying	On next sampling date		
	Mg./M²	Mg./M²	Mg./M²	%	Mg./M²	Mg./M²	Mg./M²	%
Lead								
York A	105.5	199.8	94.3		199.8	122.8	77.0	
Stayman A	108.0	202.5	94.5		202.5	127.3	75.2	
Average A	106.8	201.2	94.4	100.0	201.2	125.1	76.1	100.0
York B	138.3	214.0	75.7		214.0	164.5	49.5	
Stayman B	153.7	265.3	111.6		265.3	175.4	89.9	
Average B	146.0	239.7	93.7	99.3	230.7	170.0	69.7	91.6
York C	188.5	292.8	104.3		292.8	259.4	33.4	
Stayman C	194.5	302.0	107.5		302.0	248.5	53.5	
Average C	191.5	297.4	105.9	112.2	297.4	254.0	43.4	57.0
Arsenic (As$_2$O$_3$)								
York A	67.1	126.7	59.6		126.7	77.2	49.5	
Stayman A	66.2	124.4	58.2		124.4	75.3	49.1	
Average A	66.7	125.6	58.9	100.0	125.6	76.3	49.3	100.0
York B	80.8	152.9	72.1		152.9	94.5	58.4	
Stayman B	93.6	171.4	77.8		171.4	107.7	63.7	
Average B	87.2	162.2	75.0	127.3	162.2	101.1	61.1	95.9
York C	94.2	174.0	79.8		174.0	128.8	45.2	
Stayman C	97.4	179.2	81.9		179.2	133.5	45.7	
Average C	95.8	176.6	80.9	137.4	176.6	131.2	45.4	65.6

Table 2 shows the average amounts of lead and arsenic deposited in the six spray applications, expressed as milligrams per square meter of leaf surface. This table also shows the amounts of these elements lost during the period between sampling dates, expressed as milligrams per square meter of leaf surface. The amounts of lead and arsenic deposited and lost in treatments B and C are reported in relation to treatment A.

The graphs show the individual gains and losses in lead and arsenic at each sampling date during the period from May 28 to October 8. The graphs, with Table 2, which presents the same data in a different form, indicate that the deposition and retention of lead do not follow the same course as the deposition and retention of arsenic. For this reason, the behavior of the two will be discussed separately.

In the deposition of lead, treatments A and B were equally effective, but treatment C deposited slightly greater lead on the foliage. The relative loss of lead due to weathering was greatest from treatment A, less from treatment B, and least from treatment C, which showed a loss only 57 per cent as great as treatment A. In calculating the loss due to weathering, the losses during the period between the application of the last cover spray (July 16) and harvest (October 8) are included.

The data indicate a considerably greater deposition of arsenic on the foliage by treatments B and C than by treatment A. The losses of arsenic from the foliage paralleled the losses of lead; treatments A and B showed approximately equal losses, while treatment C showed less loss due to weathering.

In most cases there was greater deposition on, as well as greater loss from, Stayman foliage than on York Imperial. When the data were paired and analyzed statistically by Student's method (16), however, the odds against so great a difference in deposit and loss being due to chance alone were in both cases 10 to 1, which is not great enough to be considered significant.

The area measurements made in 1934 on foliage samples taken from the middle portion of the terminal growth indicated that these leaves had attained their full size by June 15, the date of the third cover spray. On the date of first cover spray the foliage of both varieties had reached approximately 78 per cent of full growth. On the date of the second cover spray the foliage of both varieties had reached 97 per cent of full growth. Thus, the first cover spray was the only one affected to any extent by the growth of the foliage. Even in this case the reduction in the amount of deposit of lead and arsenic per unit area attributable to the expansion of the leaf area was relatively small.

Lead and Arsenic on Apple Fruits— Figures 3 and 4 show the deposition and loss of arsenic and lead on the surface of the fruit during the season as influenced by variety and spray mixture. Figure 3 indicates the amounts per apple, and Figure 4 the amounts calculated on the basis of surface area and expressed in milligrams per square meter of apple surface. Calculation on the basis of each apple eliminates the dilution due to the expansion of the surface of the fruit. The apples were considered to be spheres, and the surface area was calculated from the volume determined by displacement.

Table 3 summarizes the data for apple fruits in much the same manner as Table 2 for foliage. The relative deposition of lead and arsenic on the fruit surface, expressed as milligrams per square meter, is of the same general magnitude for each of the spray mixtures; this was also true of the relative loss

TABLE 3. AVERAGE DEPOSIT OF LEAD AND ARSENIC ON FRUIT IN SIX COVER SPRAYS, AND AVERAGE LOSS OF THESE ELEMENTS BETWEEN SAMPLING DATES

Treatment	Deposit Amounts Present		Average deposit	Relative deposit	Loss Amounts Present		Average loss	Relative loss
	Before spraying	After spraying			After spraying	On next sampling date		
	Mg./M²	Mg./M²	Mg./M²	%	Mg./M²	Mg./M²	Mg./M²	%
			Lead					
York A	133.7	290.3	156.6		290.3	125.4	164.9	
Stayman A	152.9	336.0	183.1		336.0	138.1	197.9	
Average A	143.3	313.1	169.8	100.0	313.1	131.8	181.3	100.0
York B	124.0	253.6	129.6		253.6	115.5	139.1	
Stayman B	162.6	299.4	136.8		299.4	146.2	153.2	
Average B	143.3	276.5	133.2	78.4	276.5	130.9	145.6	80.3
York C	134.0	270.0	136.0		270.0	125.6	144.4	
Stayman C	191.3	333.3	142.0		333.3	179.7	153.6	
Average C	162.7	301.7	139.0	81.9	301.7	152.7	149.0	82.2
			Arsenic (As$_2$O$_3$)					
York A	42.4	121.1	78.7		121.1	39.9	81.2	
Stayman A	56.5	155.8	99.3		155.8	59.3	105.5	
Average A	49.5	138.5	89.0	100.0	138.5	45.1	93.4	100.0
York B	34.2	105.6	71.4		105.6	32.6	73.0	
Stayman B	47.3	95.9	48.6		95.9	39.5	56.4	
Average B	40.8	100.8	60.0	67.4	100.8	36.1	64.7	69.3
York C	48.2	105.5	57.3		105.5	45.9	59.6	
Stayman C	66.3	141.5	75.2		141.5	65.1	76.4	
Average C	57.3	123.5	66.2	74.4	123.5	55.5	68.0	72.8

of each of these elements from the fruit surface. The greatest deposit and the greatest loss per unit area of fruit surface occurred in treatment A. Treatment C, which contained fish oil and flotation sulfur, produced slightly greater relative deposits, and slightly greater relative losses, than treatment B, which contained skimmilk powder.

The data in Table 3 indicate an average loss greater than the average deposit for unit areas of fruit surface. This is due to the extension of the fruit surface. The average deposit and the average loss of both arsenic and lead were greater on the Stayman than on the York Imperial fruits in all cases but one.

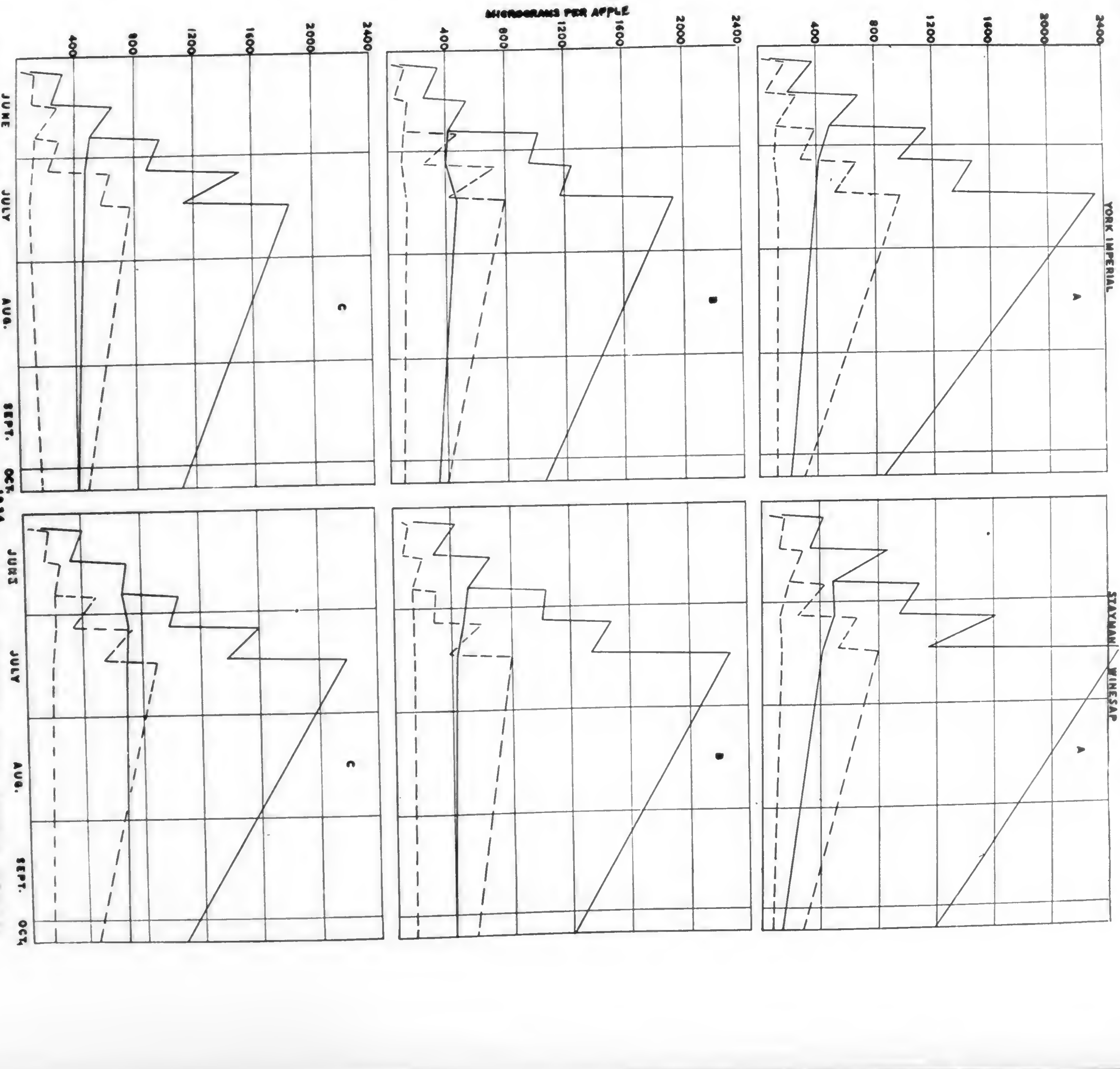

FIG. 3. LEAD AND ARSENIC (AS As$_2$O$_3$) ON FRUIT BEFORE AND AFTER SPRAY APPLICATIONS AND AT HARVEST

The solid lines represent lead, the dotted lines arsenic, calculated as micrograms per apple. The course of the deposit on the fruit of trees receiving three sprays also is shown. The first sample of fruit was taken immediately before the application of the second cover spray.

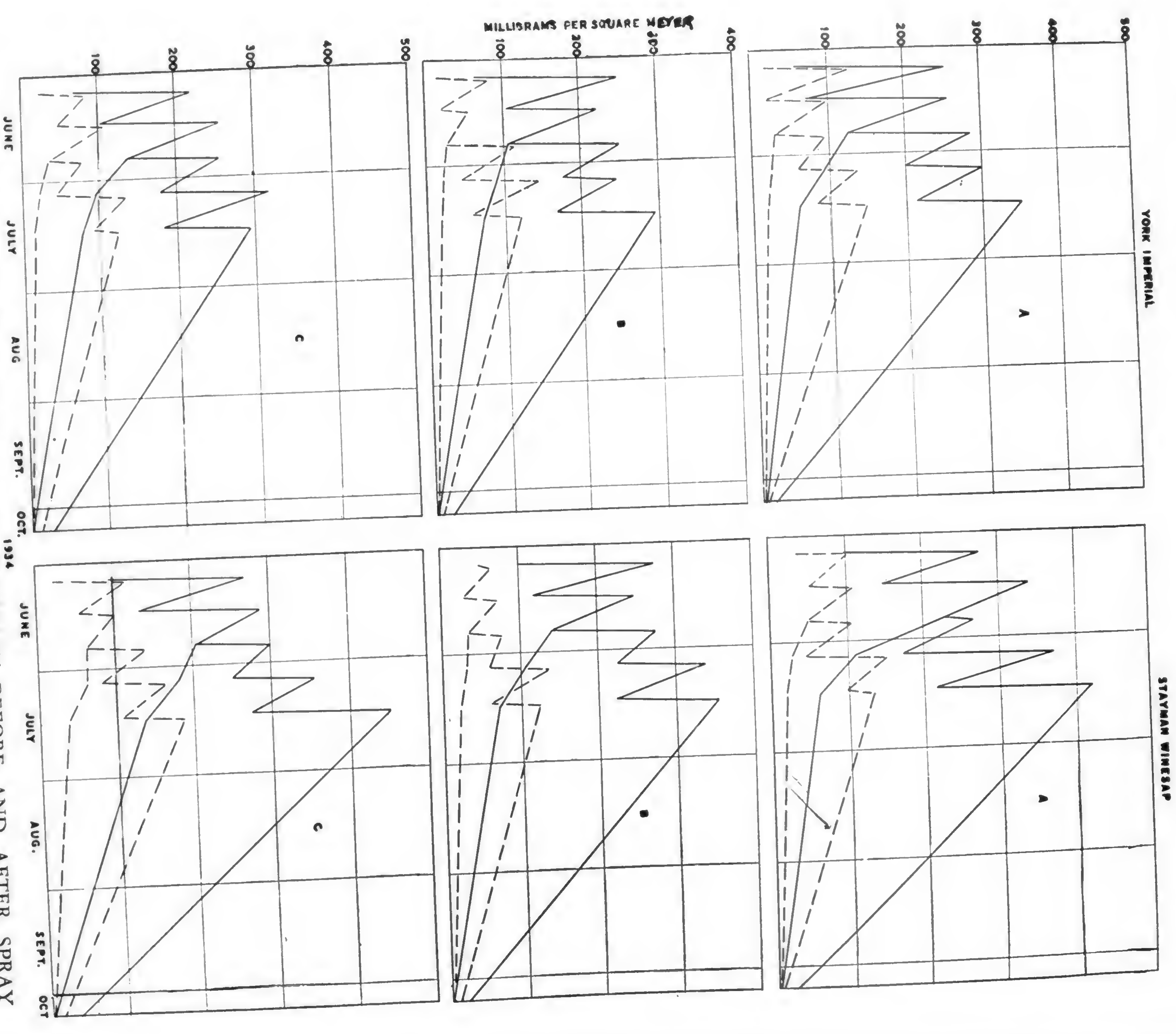

FIG. 4. LEAD AND ARSENIC (AS As_2O_3) ON FRUIT BEFORE AND AFTER SPRAY APPLICATIONS AND AT HARVEST

The solid lines represent lead, the dotted lines arsenic, calculated as milligrams per square meter. The course of the deposit on the fruit of trees receiving three sprays also is shown. The first sample of fruit was taken immediately before the application of the second cover spray.

Table 4 summarizes the data in Figure 3. The amounts of arsenic and lead present on the fruit at each sampling date are expressed as micrograms per fruit. Calculated in this way, it is possible to determine the effect of weathering on the spray deposit. The average amount deposited was considerably greater than the average amount lost. The average amount of lead and arsenic deposited on each fruit was greatest in treatment A; treatments B and C were apparently nearly equal. Treatment A showed considerably greater losses than the other two treatments. Considerably more lead per apple was lost from

TABLE 4. AVERAGE DEPOSIT OF LEAD AND ARSENIC ON FRUIT IN SIX COVER SPRAYS, AND AVERAGE LOSS OF THESE ELEMENTS BETWEEN SAMPLING DATES

Treatment	Deposit Amounts Present		Average deposit	Relative deposit	Loss Amounts Present		Average loss	Relative loss
	Before spraying	After spraying			After spraying	On next sampling date		
	Micrograms per Apple	Micrograms per Apple	Micrograms per Apple	%	Micrograms per Apple	Micrograms per Apple	Micrograms per Apple	%
			Lead					
York A	616.8	1209.8	593.0	----------	1209.8	770.6	439.2	----------
Stayman A	615.0	1305.2	690.2	----------	1305.2	825.0	480.2	----------
Average A	615.9	1257.5	641.6	100.0	1257.5	797.8	459.7	100.0
York B	576.4	1020.6	444.2	----------	1020.6	769.6	251.0	----------
Stayman B	654.2	1176.0	521.8	----------	1176.0	829.4	346.6	----------
Average B	615.3	1098.3	483.0	75.3	1098.3	799.5	298.8	65.0
York C	567.0	1065.0	498.0	----------	1065.0	769.0	296.0	----------
Stayman C	698.8	1191.6	492.8	----------	1191.6	886.0	305.6	----------
Average C	632.9	1128.3	495.4	77.2	1128.3	827.5	300.8	65.4
			Arsenic (As_2O_3)					
York A	203.2	498.2	295.0	----------	498.2	258.6	239.6	----------
Stayman A	227.6	466.2	238.6	----------	466.2	274.2	192.0	----------
Average A	215.4	482.2	266.8	100.0	482.2	266.4	215.8	100.0
York B	170.0	453.6	283.6	----------	453.6	245.4	208.2	----------
Stayman B	180.2	397.6	217.4	----------	397.6	274.6	123.0	----------
Average B	175.1	425.6	250.5	93.9	425.6	260.0	165.6	76.7
York C	211.0	421.0	210.0	----------	421.0	298.4	122.6	----------
Stayman C	249.2	511.0	261.8	----------	511.0	355.6	175.4	----------
Average C	230.1	466.0	235.9	88.4	466.0	317.0	149.0	69.0

the fruits sprayed with treatment C than from fruits sprayed with treatment B; in the case of arsenic the reverse was true. There was no consistent difference in the amounts of lead and arsenic deposited per fruit as between the two varieties studied.

Discussion of Results

Deposition-Loss on Fruits as Compared with Leaves—On the foliage, the greatest deposit of arsenic and lead was from the spray mixture containing fish oil and flotation sulfur. Lead was deposited almost equally from the spray mixture containing lead arsenate and lime sulfur alone, and the same mixture with skimmilk powder added, although arsenic was more heavily deposited from the latter. The relative losses of both lead and arsenic were greatest on the foliage sprayed with lead arsenate-lime sulfur, slightly less on that sprayed with the same mixture with skimmilk powder added, and very significantly less from the fish oil mixtures. On the fruit surfaces, both the deposit and loss were greatest in the lead arsenate-lime sulfur treatment, followed in order by the skimmilk powder and fish oil treatments.

The ratios between the amounts of lead and of arsenic present per unit of area of foliage during the period from June 26 to October 8, as compared with the same unit area of fruits, are shown in Table 5.

In all cases, the foliage-fruit ratio for arsenic is greater than the same ratio for lead. This difference may be caused by greater relative loss of arsenic than of lead from the fruits, or by smaller relative loss of arsenic than of lead from the foliage. It is evident that the former process does occur; when all treatments are averaged, 41.0 per cent of the lead deposited on the fruits by the six cover sprays was present after the last cover spray, but only 32.5 per cent of the arsenic remained. Somewhat more arsenic than lead was also lost from the foliage; the leaves retained 62.7 per cent of lead and 58.0 per cent of arsenic. This difference probably is too small to be significant.

The fact that the difference between the arsenic and lead deposits on the foliage is less than on the fruits suggests that following the decomposition of lead arsenate the arsenic is washed from the fruit surface more readily than from the leaf surface; or that the lead combines chemically with some constituent of the apple surface, possibly ursolic acid, as suggested by Cohee and St. John (2).

Influence of Variety— In 10 of 12 cases (Table 3), more lead and arsenic were deposited and lost on Stayman fruits than on York Imperial. When the toxic elements are compared on the basis of the amount present per fruit (Table 4), however, there is no significant difference between varieties. This may be because the size of the fruit was not considered in the calculation.

Relative Retention of Lead and Arsenic— In general, the amounts of lead and arsenic retained were parallel, although there were certain differences. They follow each other most closely when calculated on the basis of a unit area of fruit surface (Table 3). The ratio of arsenic trioxide/lead was greatest when no modifying material was used. The ratios for the three treatments, based on the amount present at harvest, were A, 1/3.56; B, 1/2.17; C, 1/2.24.

TABLE 5. RATIO OF THE AMOUNTS OF LEAD AND ARSENIC PER SQUARE METER OF SURFACE ON FOLIAGE TO THE AMOUNTS ON FRUIT AT VARIOUS TIMES AFTER THE LAST APPLICATION OF SPRAY

Treatment and date	Lead on foliage / Lead on fruit			Arsenic (As_2O_3) on foliage / Arsenic (As_2O_3) on fruit		
	York	Stayman	Average*	York	Stayman	Average*
	Trees receiving six cover sprays					
July 16—A	0.77	0.57	0.66	1.29	1.33	1.31
B	1.09	1.14	1.12	2.35	2.23	2.29
C	1.60	1.10	1.30	2.25	1.61	1.86
Oct. 8—A	7.34	5.65	6.34	11.02	10.31	10.59
B	8.37	7.03	7.68	11.38	9.43	10.33
C	15.98	9.39	12.21	18.14	14.72	16.20
	Trees receiving three cover sprays					
June 26—A	0.70	0.85	0.78	1.42	1.26	1.34
B	0.85	1.05	0.96	1.45	2.50	1.87
C	1.29	1.10	1.19	2.59	1.43	1.85
July 5—A	1.15	0.99	1.07	2.26	2.23	2.24
B	1.56	1.33	1.43	3.80	3.39	3.56
C	2.36	1.33	1.68	5.57	1.83	2.83
July 16—A	1.75	1.49	1.62	2.59	3.19	2.89
B	1.85	1.74	1.79	3.84	3.29	3.55
C	2.32	1.47	1.78	7.99	3.00	4.48
Oct. 8—A	24.69	27.95	25.52	20.00	35.90	25.71
B	25.37	18.59	21.68	47.00	36.95	41.41
C	22.10	10.98	14.84	30.57	30.72	30.14

* True averages calculated from original data

The most significant point, however, is the comparison of the amounts of lead and arsenic present at harvest (Table 6). This indicates the degree of resistance of each to the weathering process. The spray containing fish oil (C) gave the greatest residue at harvest, followed by the spray containing skim-milk powder (B); the lead arsenate-lime sulfur combination (A) gave the least residue at harvest. The slope of the line from the date of the last spray application (Figs. 3 and 4) indicates the rapidity of residue loss.

TABLE 6. AMOUNTS OF LEAD AND ARSENIC PRESENT ON FRUIT AND FOLIAGE AT HARVEST

Treatment	Fruit			Foliage		
	York	Stayman	Average	York	Stayman	Average
	Trees receiving six cover sprays					
	Lead—Mg./M²					
A	17.5	24.6	21.1	128.4	139.1	133.8
B	22.3	22.6	22.5	186.7	158.9	172.8
C	27.8	36.7	32.3	444.3	344.6	394.5
	Arsenic (As_2O_3)—Mg./M²					
A	6.2	5.9	6.1	68.3	60.8	64.6
B	8.4	10.0	9.2	95.6	94.3	95.0
C	11.8	15.6	13.7	214.1	229.7	221.9
	Trees receiving three cover sprays					
	Lead—Mg./M²					
A	3.5	2.2	2.9	86.4	61.5	74.0
B	5.1	6.1	5.6	129.4	113.4	121.4
C	6.8	12.8	9.8	150.3	140.5	145.4
	Arsenic (As_2O_3)—Mg./M²					
A	1.8	1.0	1.4	36.0	35.9	36.0
B	1.5	1.9	1.7	70.5	70.2	70.4
C	2.8	2.9	2.9	85.6	89.1	87.4

Relation between Spray Deposit and Fruit Growth—The relation between the amounts of lead and arsenic on the fruit and on the foliage is of interest, since it illustrates the effect of the growth of the fruit surface. After the application of the last cover spray, on July 16, the levels of lead on the three treatments were approximately the same per unit of surface area on both fruits and foliage (Table 7).

The ratios in Table 5 show that on the trees receiving six cover sprays there was from 6 to 12 times as much lead per unit area on the foliage as on the fruit at harvest. The relative amounts of arsenic on the two surfaces showed an even wider spread, possibly due to a higher ratio after the last cover spray. During the period considered, which was approximately 11 weeks, the fruit surfaces expanded to about 8½ times their original area, while the leaf surfaces showed no increase. On trees receiving three cover sprays the amount of lead at harvest per unit area of leaf surface was from 14 to 25 times larger than per unit area of fruit surface. On these same trees the amount of arsenic was from 25 to 41 times larger on the leaf surfaces than on the fruit surfaces, both being expressed as amounts per square meter of surface.

During the 14 weeks between the last spray application and harvest the area of the fruits increased, on the average, about 13½ times, while the area of the leaves did not increase. The difference in loss of spray deposits from fruits as compared with leaves was greater than that which might be expected from growth alone, indicating that the materials weathered more rapidly from the fruits. This is due in part to the nature of the apple surface and in part to its expansion, which causes a mechanical loosening of the deposits. However, the amounts of lead and arsenic on fruits and foliage, when calculated on the basis of the amount present per fruit and per leaf, show little difference at any time during the season (Table 8). This indicates that the growth of the fruit surface is the major factor in the reduction of the deposits.

TABLE 7. AMOUNTS OF LEAD AND ARSENIC PRESENT ON FRUIT AND FOLIAGE AFTER LAST COVER SPRAY, JULY 16

Treatment	Fruit			Foliage		
	York	Stayman	Average	York	Stayman	Average
	Trees receiving six cover sprays					
	Lead—Mg./M²					
A	352.4	422.7	387.6	270.4	241.3	255.9
B	296.8	355.3	326.1	324.0	405.8	364.9
C	296.0	453.0	374.5	473.4	496.5	485.0
	Arsenic (As_2O_3)—Mg./M²					
A	147.4	133.9	140.7	190.0	178.7	184.4
B	122.4	124.6	123.5	287.8	278.4	283.1
C	124.0	186.4	155.2	278.7	299.9	289.3
	Trees receiving three cover sprays					
	Lead—Mg./M²					
A	59.1	63.0	61.1	103.4	94.3	98.9
B	73.2	72.6	72.9	133.9	126.5	130.2
C	77.3	136.5	106.9	179.0	201.3	190.2
	Arsenic (As_2O_3)—Mg./M²					
A	20.0	20.9	20.5	51.7	66.7	59.2
B	20.3	23.7	22.0	78.0	77.9	78.0
C	15.6	37.0	26.3	124.6	110.8	117.7

Losses of Lead and Arsenic through Weathering— The influence of rainfall on the weathering of spray residue from leaf and fruit surfaces was apparently not highly important when this method of sampling (from the bottom limbs only) was followed. No consistently greater loss was observed when rain fell than during periods free from rain.

TABLE 8. RATIO OF THE AMOUNTS OF LEAD AND ARSENIC PER 100 LEAVES, IN MILLIGRAMS, TO AMOUNTS OF LEAD AND ARSENIC PER FRUIT, IN MICROGRAMS AT VARIOUS TIMES AFTER THE LAST APPLICATION OF SPRAY

Treatment and date	Lead on foliage / Lead on fruit			Arsenic (As_2O_3) on foliage / Arsenic (As_2O_3) on fruit		
	York	Stayman	Average	York	Stayman	Average
Trees receiving six cover sprays						
July 16—A	0.13	0.16	0.15	0.23	0.26	0.25
B	0.18	0.23	0.21	0.46	0.57	0.51
C	0.27	0.28	0.27	0.31	0.43	0.37
Oct. 8—A	0.10	0.08	0.09	0.14	0.14	0.14
B	0.13	0.10	0.12	0.18	0.14	0.15
C	0.25	0.24	0.24	0.28	0.36	0.32
Trees receiving three cover sprays						
June 26—A	0.13	0.14	0.14	0.27	0.21	0.24
B	0.16	0.19	0.18	0.29	0.45	0.35
C	0.29	0.25	0.27	0.58	0.32	0.42
Oct. 8—A	0.24	0.34	0.28	0.18	0.42	0.27
B	0.25	0.23	0.24	0.46	0.47	0.47
C	0.25	0.17	0.20	0.38	0.47	0.43

Influence of Frequency of Spraying on the Deposition and Retention of Lead and Arsenic

The foregoing studies indicate that the variety of fruit and the composition of the spray mixture have a decided effect on the amounts of lead and arsenic deposited and retained on the surfaces of apple foliage and fruits. They also indicate that the relative amounts of lead and arsenic on the plant surfaces are not the same throughout the season, the losses of arsenic being greater than the losses of lead. However, the greatest single factor affecting the loss of spray deposits appears to be the expansion of the plant surfaces due to growth. During the period of spray applications, which was 11 weeks, the surface area of the fruits increased about 8½ times. During the period between the last spray and harvest, which was 14 weeks, the fruits increased in surface area about 13½ times. The average area of the leaves did not increase appreciably during the period of spraying and subsequently; the foliage practically attained its maximum area at the time of the second cover spray.

From measurements of the fruits analyzed during the season of 1934, a chart was constructed showing the time of increase of the surface area of apple fruits. It appeared that it should be possible to choose dates for spray applications which would give a more uniform coverage of lead and arsenic upon the fruit surfaces during the season than present spraying schedules. The area of apple fruits increases very rapidly during the early stages of its development, causing a rapid lowering of the concentration of lead and arsenic per unit area of surface. The increase in surface area becomes progressively less as the season advances so that the loss of deposit from this cause becomes less important as the fruits become more mature. Hamilton (7) found a relationship between the increase of surface area and the reduction of deposits of arsenic trioxide. Similarly, Lathrop and Sazama (10) found the larvacidal efficiency of coatings of lead arsenate to be lowest during the early period of rapid expansion of the fruit surface. Steiner *et al.* (15) have demonstrated the value of supplementary cover sprays early in the season.

Object of the Experiments of 1935—It was reasoned that, instead of applying the sprays at uniform intervals during the period of attack, as is commonly done, more efficient insect control with stomach poisons might be secured by applying the sprays at more frequent intervals during the early part of the season, and spacing them farther apart during the later period of less rapid expansion.

Several workers, including Percival and Potter (12), have found that the deposits of spray residues decrease from the bottom to the top of the tree. This is partly because the operator fails to cover the upper branches thoroughly, and partly because the upper portions of the trees are more exposed to weathering than the lower branches. The object of this investigation was to maintain a more uniform deposit of lead and arsenic upon the fruit by timing the applications to coincide more nearly with the growth curve of the surface of the apples, and by supplementary sprays applied only to the tops of the trees.

Plan of the Experiments

Three groups of five trees each were selected in the College orchard. All were Stayman, 18 years old, and vigorous. The spray treatment through the petal fall application was uniform in all groups. The first experimental spray was applied on June 4.

All groups received the same spray mixture, consisting of lead arsenate 3 pounds, flotation sulfur 5 pounds and cold-pressed menhaden fish oil 1 quart per 100 gallons. The five trees in Group D were sprayed at intervals of 14 days, each tree receiving thorough coverage. The five trees in Group E were sprayed at the same time and in the same manner as those in Group D. In addition, the tops of these trees only were sprayed at 14-day intervals, beginning seven days after the first application, so that the top applications alternated with the complete coverage. The trees in Group F received complete applications at intervals of 4, 10, and 20 days following the first application, which was given at the same time as the initial application to Groups D and E. This spacing was the result of a study of the growth curve for the fruit of this variety in 1934, based on the measurement of the relatively small number of fruits analyzed that season.

Duplicate samples of 20 apples each were collected from all trees before and after each spray application and at intervals thereafter until August 20. The samples taken after this date were duplicate lots of 10 apples each. All apples in each sample were measured transversely and vertically with micrometer calipers and the surface area calculated, the mean of these two diameters being considered to be the diameter of a sphere. Other apples of Stayman and other varieties were measured in the same way at intervals throughout the growing season.

The apples for analysis were picked without handling by impaling them on steel pins, and were carried to the laboratory in special boxes designed to keep each fruit from coming in contact with the container or with the other apples. The samples were weighed and the lead determined by the method of Frear and Haley (4). The arsenic was determined on an aliquot of the same solution by the Gutzeit method (1).

Weather—Table 9 gives the amounts of rainfall and the mean monthly temperatures during the growing season of 1935 as compared with normal. The mean monthly temperatures were very near normal. The precipitation was slightly less than the 50-year averages of this station.

TABLE 9. MONTHLY MEAN TEMPERATURES AND INCHES OF RAINFALL DURING 1935 GROWING SEASON AT STATE COLLEGE, PA., COMPARED WITH NORMAL

	Mean temperature		Rainfall, Inches	
	1935	Normal	1935	Normal
May	55.6	58.78	3.59	4.12
June	66.5	66.39	3.78	4.27
July	73.5	76.20	5.83	3.84
Aug.	69.2	68.49	1.44	3.57
Sept.	60.9	62.65	2.40	2.98
Oct.	52.5	51.07	2.11	2.64
Total			19.15	21.42
Average	63.0	63.93		

Experimental Results

Figure 5 is a smoothed growth curve of the surface area of Stayman fruit during the season of 1935. In preparing this curve, certain slight irregularities have been smoothed out. The upper line represents the average areas of the fruits picked from the tops of the trees; the lower line represents the average areas of the fruits picked from the lower limbs. During the months of July, August, and September, the difference in the size of the fruits from the top and from the bottom was very noticeable. The data used in constructing these areas were collected partly by measuring the samples which were analyzed and partly by measuring fruits of the same variety grown in the same orchard under similar conditions. The growth curves in Figure 5 involved the measurement of approximately 5,000 apples. The curves for other varieties which were measured at the same time show a similar trend, the early varieties showing more rapid growth earlier in the season, as would be expected.

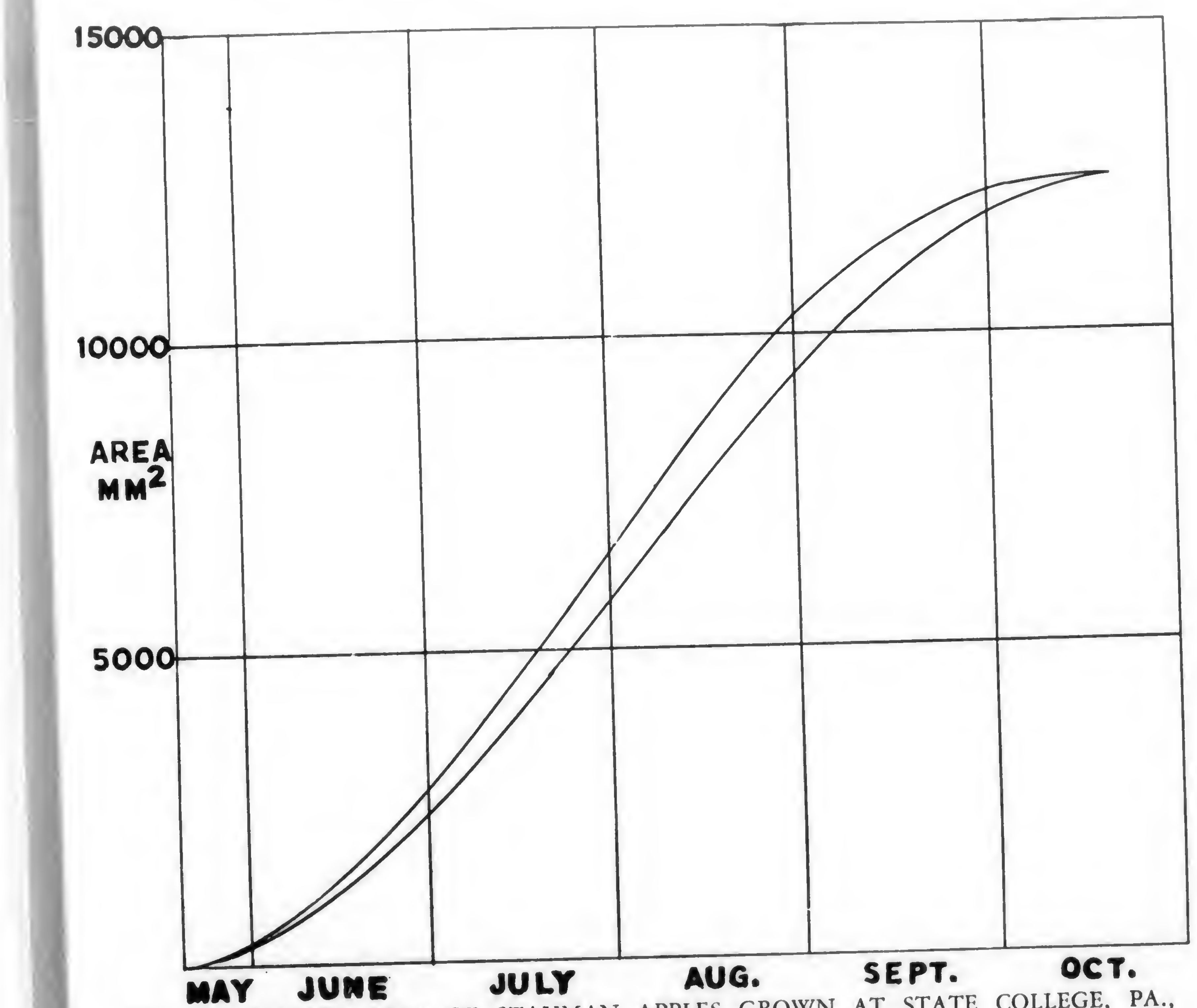

FIG. 5. SURFACE AREA OF STAYMAN APPLES GROWN AT STATE COLLEGE, PA., DURING THE SEASON OF 1935

The upper line represents the area of fruits from the tops of the trees; the lower line the areas of the fruits from the bottoms of the trees.

The determinations of lead and arsenic trioxide are reported in Figures 6 and 7. In Figure 6, the results are calculated as milligrams of lead (solid line) and milligrams of arsenic trioxide (broken line) per square meter of apple surface. In Figure 7, the amounts of lead and arsenic trioxide are calculated as micrograms per apple, the broken lines representing arsenic trioxide and the solid lines lead. On the left half of both figures are given the amounts found upon apples picked from the top of the trees; on the right, the amounts found on fruits picked from the bottom of the trees.

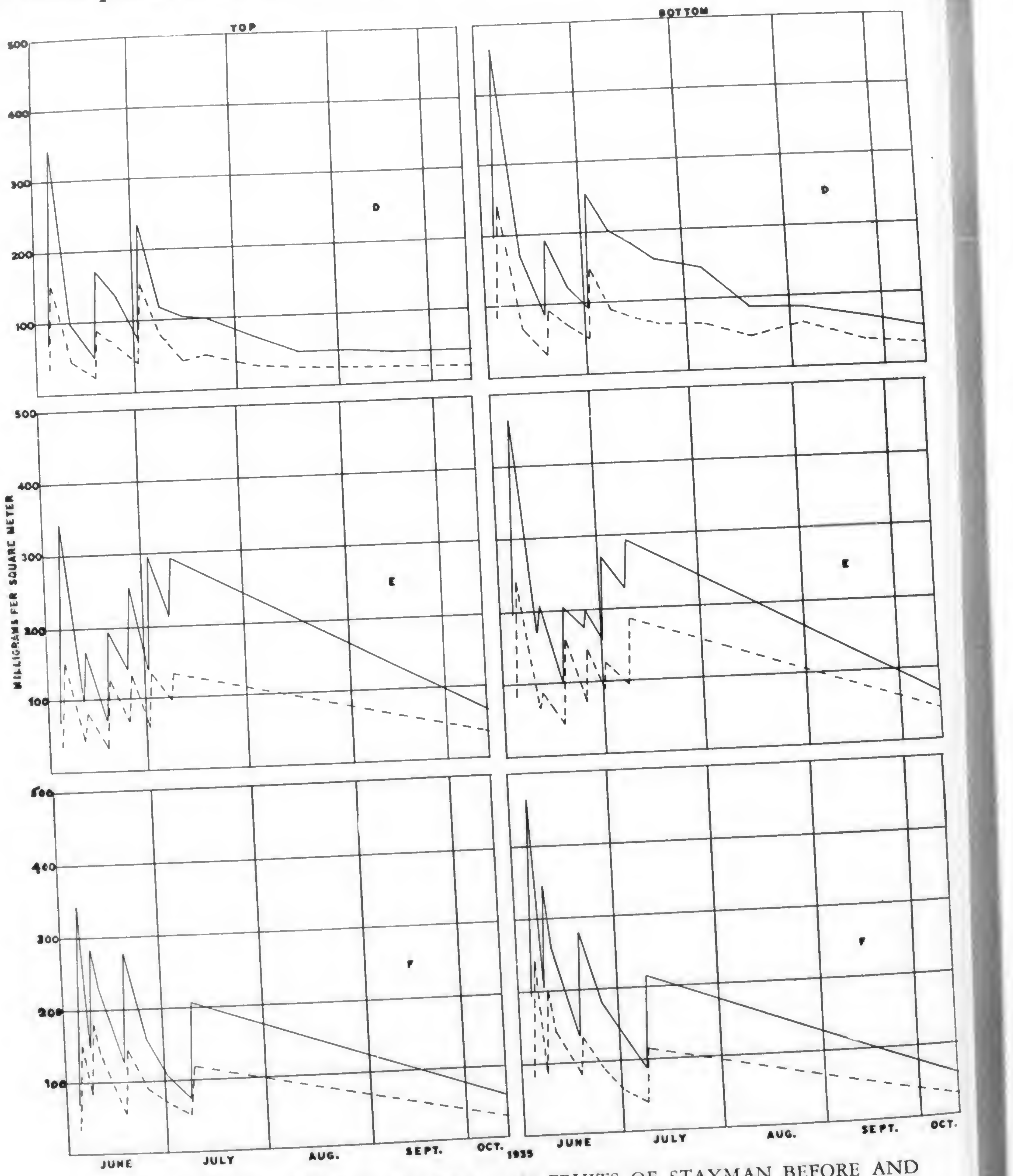

FIG. 6. LEAD AND ARSENIC (AS As_2O_3) ON FRUITS OF STAYMAN BEFORE AND AFTER SPRAY APPLICATIONS AND AT HARVEST

Calculated as milligrams per square meter of apple surface. The solid lines represent lead, the dotted lines arsenic. Season of 1935.

Table 10 shows the amounts of lead and arsenic deposited in each of the cover sprays. These are calculated as milligrams of lead and arsenic trioxide per square meter of apple surface and represent the differences in amounts found before and after spraying. Table 11 gives the amounts of lead and arsenic trioxide found on the fruit surfaces immediately after the last cover spray application, on July 9, and at harvest, on October 21. The results are expressed in the same manner as in the preceding table.

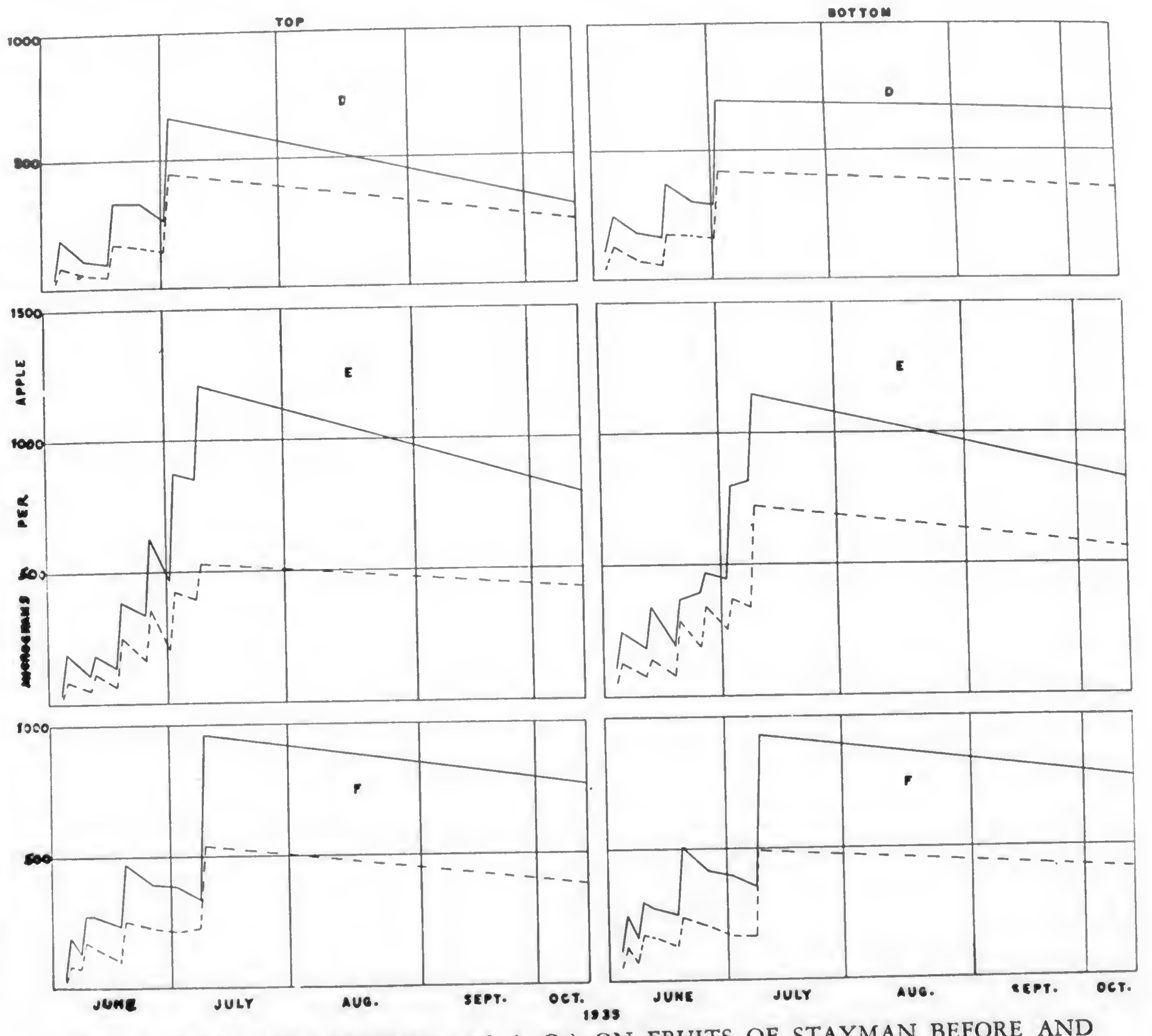

FIG. 7. LEAD AND ARSENIC (AS As_2O_3) ON FRUITS OF STAYMAN BEFORE AND AFTER SPRAY APPLICATIONS AND AT HARVEST

Calculated as micrograms per apple. The solid lines represent lead, the dotted lines arsenic. Season of 1935.

Figure 8 shows the mean amounts of lead present on the fruit during the periods between spray applications. These are calculated as milligrams per square meter of apple surface for fruits from both the top (left) and the bottom (right) of the trees receiving each treatment. The figures were obtained by averaging the amounts present after one application and before the next. The figures for arsenic so closely resemble those for lead that they are not presented here. The black portion of the chart indicates the periods between application dates during which the mean deposit of lead exceeded 125 milligrams per square meter. It also indicates the extent to which this arbitrary minimum effective deposit was exceeded.

TABLE 10. AMOUNTS OF Pb AND As_2O_3 DEPOSITED IN EACH COVER SPRAY APPLICATION EXPRESSED AS MILLIGRAMS PER SQUARE METER

Date	Treatment D		Treatment E		Treatment F	
	Top	Bottom	Top	Bottom	Top	Bottom
			Lead			
June 4	274.1	269.9	274.1	269.9	274.1	269.9
June 8			70.1	37.9	137.3	139.5
June 11			123.6	105.6	152.7	143.8
June 18	121.1	106.2	112.8	21.6		
June 25			157.4	113.1		
July 2	160.9	161.6	79.7	65.2	130.7	128.0
July 9						
Average	185.4	179.2	185.0*	182.8*	173.7	170.3
			Arsenic			
June 4	116.0	158.3	116.0	158.3	116.0	158.3
June 8			36.1	20.9	96.9	111.8
June 11			94.9	116.4	124.1	50.1
June 18	65.4	61.3	74.3	62.1		
June 25			73.2	35.7		
July 2	111.8	98.4	34.7	90.7	67.6	74.7
July 9						
Average	97.5	106.0	94.7*	103.5*	101.2	98.7

* These averages are for the complete applications: the average amounts of Pb in the applications to the tops alone were for the top and bottom of the trees 87.5 and 41.6 mg. respectively, and for As_2O_3 48.4 and 57.9 mg. per M^2.

TABLE 11. AMOUNTS OF Pb AND As_2O_3 PRESENT ON THE FRUIT SURFACES AFTER THE LAST COVER SPRAY AND AT HARVEST EXPRESSED AS MILLIGRAMS PER SQUARE METER

	Treatment D		Treatment E		Treatment F	
	Top	Bottom	Top	Bottom	Top	Bottom
			Pb			
After last cover spray application	118.1*	201.2*	292.3	292.3	207.2	215.0
At harvest	43.4	53.0	68.0	65.6	58.7	57.7
(Calculated as grain per lb.)	.023	.040	.044	.045	.038	.038
			As_2O_3			
After last cover spray application	78.8*	89.3*	131.3	184.7	118.6	115.7
At harvest	25.0	23.9	37.0	44.5	29.3	31.1
(Calculated as grain per lb.)	.013	.018	.024	.031	.019	.021

* Samples from treatment D received the last cover spray on July 2; the others on July 9. All samples were taken on July 9.

It should be emphasized that this "minimum effective deposit" of 125 milligrams per square meter of lead is a purely arbitrary figure, which may be subject to adjustment under different levels of codling moth attack. However, records from spraying experiments with apples in Adams county, when compared with the deposit figures developed in this study, seem to indicate that under southern Pennsylvania conditions this minimum deposit of lead is the least that should be present on the fruit during the period of codling moth attack. An extremely small codling moth population in the orchard in which this experiment was conducted made it impossible to measure the efficiency of control. This phase of the investigation will receive further study.

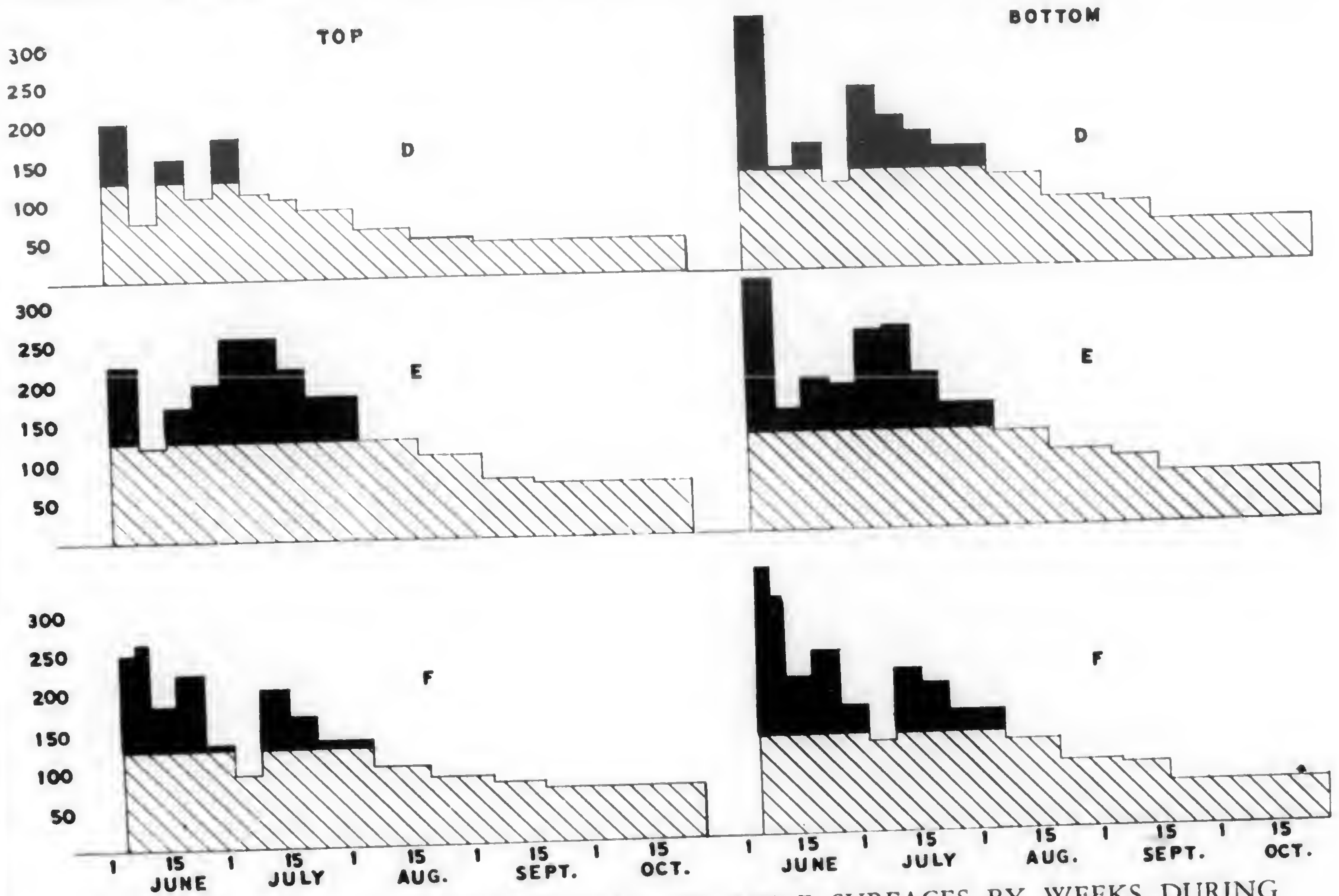

FIG. 8. AVERAGE DEPOSIT OF LEAD ON APPLE SURFACES BY WEEKS DURING THE GROWING SEASON OF 1935

Calculated as milligrams per square meter of apple surface. The black area indicates deposits above 125 mg. per square meter.

Discussion of Results

The data presented in Figures 6 and 7 show the actual amounts of lead and arsenic present before and after each spray application, and (for treatment D) at various times following the last spray. The amounts of lead and arsenic present at harvest are shown for all treatments. In treatment D, the amounts of lead and arsenic on the fruit surfaces varied widely, even during the period of spray application. For example, the first spray application resulted in a deposit of lead on the fruit at the bottom of the tree of 465 mg. per square meter. Two weeks later, this had decreased to 84 mg. This rapid loss was paralleled on the fruit at the top of the tree during the same period, from June 4 to June 18. Figure 6 shows that during this period the area of fruit surface was approximately tripled by growth. This, alone, would account for a two-thirds loss in deposit.

Figures 6 and 8 show that the fruits in treatment D were inadequately protected (if 125 mg. per square meter is considered as the minimum effective deposit) during much of the spraying period by applications spaced 14 days apart. This was particularly true of fruits in the upper parts of the trees.

Deposits on Top Fruits— The applications to the tops of the trees in treatment E produced a much more uniform deposit over the entire tree. Figure 8, however, shows that even with such applications the deposits on the top fruits during the period between June 11 and June 18 fell below 125 mg. per square meter. This may have been due, in part, to the large percentage of increase in fruit area from June 4 to June 11; this may have reduced the deposit to such a low point that the top application of June 11 failed to raise it sufficiently above the 125 mg. level.

During the period July 2 to July 18 the top fruits carried a deposit which was probably considerably in excess of the amount required for protection. The growth curve (Fig. 8) suggests that during this period expansion of the fruit surfaces was not rapid enough to require a 7-day spacing of sprays. In this respect, treatment E may, therefore, be called a wasteful procedure.

Treatment F corrected one fault common to both treatments D and E. The second cover spray, which was applied on June 8, four days after the first cover, and the third cover spray applied 10 days later on June 18, maintained the spray coating above the level of 125 mg. per square meter during the early period of rapid growth. The growth factor, however, prevented an actual build-up of deposits from the spray applications. In consequence, the spray application of June 18 gave adequate protection only until about July 1. The schedule used in treatment F, which was based on the few fruits measured in 1934, called for an interval of 20 days between the third and fourth applications. The more extensive measurements of fruits in 1935 showed this interval to be too great.

In south central Pennsylvania the heaviest attack by codling moth larvae of the first generation falls between June 10 and July 10, the exact time of peak damage being determined by seasonal conditions. It is evident that none of the three spraying schedules used in this experiment provided adequate protection throughout this period to both top and bottom fruit. The results with treatments E and F, however, indicate that a combination of these two schedules might provide protection without producing excessively heavy deposits.

The creation of a heavy deposit early in June is essential to insure adequate amounts later. Because the fruits grow and deposits are reduced faster in the tops than at the bottoms of the trees, some supplementary top spraying would appear to be advisable. Figure 8 suggests that four cover sprays, properly spaced, will protect the fruit from heavy attack by first generation codling moth larvae until about mid-July. At that time a final application to the tops alone may carry the protection well into August without producing residues difficult to remove at harvest.

Close Spacing of Early Sprays— Treatment F shows the desirability of close spacing of the first and second cover sprays. Treatment E shows that applications to the tops alone would not be successful as early as the second cover spray because of the rapid increase in surface area of the fruits. Figure 6 shows that the first cover spray of June 4 raised the lead level to nearly 350 mg. per square meter, but that it dropped to 150 mg. in 4 days, and to 100 mg. in 7 days. Figure 5 shows that at this period, June 8 to June 18, the fruits may be expected to double in surface area in approximately six days. To be most effective, therefore, a second complete application should follow the first within five days.

On June 18, when the third cover spray was applied in treatment F the lead level had dropped nearly to 125 mg. per square meter (Fig. 6). To provide a margin of safety, a maximum interval of nine days should elapse between the second and third applications. A top application should not be attempted at this time, for the complete application to treatment F failed to provide adequate protection beyond June 30.

Growth of Apple Surface— Figure 5 indicates that the surface area of the fruits doubled between June 18 and July 4. The application of June 18 raised the lead level on top fruit in treatment F to 278 mg. per square meter. On July 2 this had dropped to 107 mg., although growth alone should have brought it to only 139 mg. by July 4. Weathering accounted for the remainder. The spacing of applications must take this into account when it is desired to lengthen the time between sprays. Basing judgment on these considerations, the fourth spray application should follow the third at an interval not greater than 13 days. A complete application at this time would correct the condition apparent in treatment F (Fig. 8), in which a greater delay in the fourth application resulted in a period of inadequate protection, particularly in the tops of the trees.

Growth doubled the surface area of the top fruit between July 1 and July 25. During such an extended period weathering may constitute a large factor in the reduction of spray deposits. The application of July 2 in treatment D raised the lead level to 236 mg. per square meter; yet on July 9, after 2.65 inches of rainfall, this was reduced to 118 mg. Figure 7 (treatment D, top) indicates that this loss is due to weathering, since the slope of the line from July 2 to harvest on these fruits is much steeper than on the bottom fruits. Theoretically, a fourth spray, on July 1, should produce a lead deposit close to 300 mg. per square meter, which growth would reduce to 150 mg. by July 25. Since rainy weather during this period may be expected to result in much more rapid loss, particularly from the top fruit (Fig. 6, treatment D), a fifth application somewhat in advance of this date, to the tops alone, probably would be advisable in the face of continued codling moth attack. From the growth curve it appears that this application, if made 17 days after the fourth cover spray, would extend the protection another three weeks.

The above discussion is not a recommendation for spraying practice. It is an attempt to indicate the importance of the growth of the fruit as a factor in the reduction of spray deposits, and to show how this effect may be overcome through changes in the timing of the spray applications. Further work in orchards with high codling moth populations will be carried out during 1937 to serve as a basis for recommendations to fruit growers.

Table 11 shows that while the deposits of lead and arsenic present on July 9 reflect the spray schedules applied in the various treatments, the differences are minimized by growth plus weathering during the long interval between this date and harvest. With all the three schedules employed, the fruits required washing to bring the residues below the prevailing tolerances of .018 grain per pound of lead and .010 grain per pound of arsenic trioxide. However, even in treatment E the level of these residues was such as not to make the washing problem difficult.

Summary and Conclusions

Samples of fruit and foliage were taken from Stayman and York Imperial apple trees before and after each spray application during the season of 1934 and at harvest. Three spray mixtures were applied: A, containing lead arsenate and lime sulfur; B, lead arsenate, lime sulfur, and skimmilk powder; and C, containing lead arsenate, flotation sulfur, and fish oil. The samples were analyzed for arsenic and lead. Besides showing the relative course of the deposit and retention during the season, the following conclusions were reached:

1. The deposition of lead on the foliage did not follow the same course as the deposition of arsenic. The greatest deposition of both elements was from spray mixture C, and least from mixture A.
2. Losses of arsenic and lead from the foliage were greatest from spray mixture A, least from spray mixture C.
3. There was greater deposition of arsenic and lead on Stayman foliage than on York Imperial; losses were also greater from Stayman foliage.
4. The foliage had attained, on the average, 78 per cent of its full size at the time of the first cover spray (May 28) and 97 per cent of full size at the second application (June 6); hence the growth of the foliage did not greatly change the concentration of lead and arsenic per unit area of leaf surface.
5. On the surface of the fruit, the greatest deposit and the greatest loss per unit area occurred with spray mixture A. Mixture C gave greater deposit and slightly greater loss than mixture B.
6. The greatest deposit of arsenic and lead, when calculated as micrograms per fruit, was secured with spray mixture A, and almost equal deposits with mixtures B and C. Treatment A produced a deposit more readily lost than mixture B. The least loss per fruit was from those sprayed with mixture C.
7. Immediately after the application of the last cover spray the amounts of lead and arsenic per unit area were not widely different on fruit and foliage, but at harvest there was 10 to 16 times more arsenic on the foliage than on the fruits, and 6 to 12 times more lead, on trees receiving six cover sprays. On trees receiving three cover sprays, the last being three weeks earlier in the season, there was from 25 to 41 times more arsenic on the foliage than on the fruits, and from 14 to 25 times more lead. This was due in part to the more rapid growth of the fruit surfaces. The difference in the relative amounts of arsenic and lead may be due to the chemical combination of the lead with certain constituents of the waxy covering of the fruit.
8. The amount of rainfall between spray applications apparently had little effect on the rapidity of the weathering of the spray residues, when samples were taken only from the bottoms of the trees.

A second investigation was concerned with the effect of spacing spray applications and of supplementary sprays to the tops alone upon the uniformity and adequacy of spray coatings of lead arsenate upon fruits during the period of first brood codling moth attack. Eighteen-year-old Stayman trees were sprayed with lead arsenate 3 pounds, menhaden fish oil 1 quart, and flotation sulfur 5 pounds per 100 gallons of water. Five trees each were sprayed according to the following schedules, all applications beginning on June 4, 1935.

Treatment D. Three applications to the whole tree at intervals of 14 days.

Treatment E. Three applications to the whole tree at intervals of 14 days, plus three applications directed at the tops alone, given seven days after each of the complete applications.

Treatment F. Four applications to the whole tree at intervals of 4, 10, and 20 days, respectively. These intervals represented the length of time required for the doubling of the surface area of the fruits, as shown by a growth curve based on measurements of apples analyzed in 1934.

Fruit samples were taken from the top and bottom limbs of trees in each treatment before and after each spray application, at intervals after the final application, and at harvest. These were measured and analyzed for lead and arsenic. Figures were prepared showing the levels of these elements maintained throughout the season in milligrams per square meter of apple surface.

The 14-day spacing of spray applications (treatment D) resulted in inadequate coverage, particularly during the early season of rapid fruit growth. This result was especially noticeable in fruits taken from the tree tops.

In treatment E, the addition of supplementary applications to the tops alone maintained spray coatings in the tops at a level approximately equal to that of the bottoms of the trees, except early in June, when the interval between applications was too great. Later, these supplementary sprays produced spray coatings considerably in excess of the minimum amount considered necessary for protection.

Spray applications based on the calculated growth curve of fruits taken from the bottoms of the trees in 1934, as applied in treatment F, maintained adequate spray coatings during the period of rapid growth in June, but these coatings became deficient during the 20-day interval between the last two sprays. Construction of growth curves based on measurements of large numbers of apples in both the tops and bottoms of the trees in 1935 showed this interval to be too great, particularly for fruits in the tree tops.

The results suggest the following conclusions, *if it is considered that the minimum amount of lead residue on the fruit necessary for protection is 125 milligrams per square meter of fruit surface:*

1. The loss of lead and arsenic is greater from the fruits at the tops of the trees than from those at the bottoms, probably because of the greater weathering at the tops, and because these fruits grew more rapidly earlier in the season.
2. To afford adequate protection by the use of lead arsenate during the period of most rapid increase in the surface area of apple fruits, the interval between the first and second codling moth cover sprays should not exceed five days.
3. A 9-day interval may be expected to elapse between the second and third codling moth cover sprays before spray coatings become inadequate for protection. This extension of the time interval is made possible by the gradual slowing up in rate of surface area increase.
4. The fourth cover spray should be applied 13 days after the third.
5. Under conditions of heavy codling moth attack a fifth spray, applied to the tops only, 17 days after the fourth, will extend the period of protection into early August without producing residues difficult to remove at harvest.
6. The thorough application of cover sprays timed as given above will maintain a lead deposit on apple fruits greater than 125 milligrams per square meter during the period of attack by first and early second brood codling moth larvae. It is thought that this concentration of spray deposits will prove

adequate under the prevailing conditions of heavy codling moth attack in southern Pennsylvania. *However, recommendations for orchard practice must await further testing of this proposed "growth schedule" of codling moth spray applications.*

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Our observation that the concentration of blood sugar influences the pharmacological effects of alcohol may offer some explanation of the alleged idiosyncrasies in human reactions to alcohol. It affords a new factor that must be taken into consideration in any experimental study of the pharmacology of alcohol. Our technique of determining doses under conditions of equilibrium affords a method of making precise quantitative measurement of the toxicity of alcohols from various sources.

HOWARD W. HAGGARD
LEON A. GREENBERG
YALE UNIVERSITY

THE OPERCULAR APPROACH TO THE PITUITARY[1]

SURGICAL techniques for the removal of the pituitary gland of teleosts have been described by Parker[2] in the catfish, *Ameiurus*, and by Matthews[3] in the killifish, *Fundulus*. These techniques are not quite satisfactory in experiments to be carried on over long periods of time, inasmuch as the operated animals survived only for a short time; the catfish surviving only two days, and the killifish several weeks. A new approach to the teleost hypophysis whereby tissue injury is reduced to a minimum has been worked out by the author with more satisfactory results.

(1) THE CATFISH

Parker's method of hypophysectomy consisted in making a U-shaped cut through the gular membrane from the opercular opening on one side to that on the opposite side of the animal, so that the lower jaw was completely separated from the gills. Having accomplished this initial opening, the operation was completed by puncturing a hole in the parasphenoid and sniping off the gland. This method involves a two-inch incision which may be avoided in the following manner. The catfish is wrapped in a wet cloth and placed on its back under a binocular microscope. The

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(2) THE KILLIFISH

Matthews's method of hypophysectomy involved a sub-oral route, since he first made a V-shaped incision through the branchiostegal membrane with the base of the V at the tip of the tongue. The tongue was then pulled sufficiently ventral to expose the region of the pituitary. My operations with the sub-oral route proved unsatisfactory mainly because of the size of the initial incision, and the necessary cutting of a large blood vessel running along the ventral surface of the lower jaw. These two difficulties may be avoided by the opercular approach. The fish, first immobilized by immersion in cracked ice, is placed on its left side under an operating microscope. The operculum is elevated and held by a single retractor. The first and second gill arches are separated, thus exposing a wide space directly below which the hypophysis is located. A three-millimeter incision is made through the epithelial membrane slightly lateral to the mid-line in order to avoid a median artery. A hole is drilled with a trephine drill (1 mm in diameter) and the gland sucked up in a pipette. The wound is not closed by a suture because of the proximity of the median artery, but the circular piece of bone excised by the drill may be replaced to close the wound. The operation may be performed within five minutes and if done with sufficient care is a totally bloodless operation. The animal is placed in salt water for a day and then transferred to running tap water, as Matthews recommends, or it may be placed directly in tap water at room temperature.

This approach to the pituitary gland under the operculum and between the first and second gill arches may prove satisfactory for other teleost fishes.[4]

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[1] Aided in part by a grant from the National Research Council Committee on Problems of Sex administered by F. L. Hisaw.

[2] G. H. Parker, *Jour. Exp. Zool.,* 69: 199, 1934.

[3] S. A. Matthews, *Biol. Bull.,* 64: 315, 1933.

[4] Smith, Burr and Ferguson (*Endocrin.,* 19: 409, 1935), describe an orbital approach for hypophysectomy in the goldfish. These authors state, however, that their operative procedure involves enucleation of the right eye, severe traumatization of the interorbital plates, invariable rupture of an artery, blind groping for the hypophysis, traumatization of the hypothalamus and successful extirpation in one of seventeen cases.

THE APPLICATION OF SPRAYS TO EXPANDING PLANT SURFACES[1]

DURING the season of 1934, the authors had occasion to make a series of measurements of the surface areas of apple fruits grown in the college orchard at State College, Pa. This work was done in connection with a study of the deposition and retention of lead and arsenic trioxide on the fruit and leaf surfaces of two varieties of apples sprayed with three spray mixtures. From these measurements and from the chemical analyses of the fruit surfaces for the two toxic elements, it was apparent that the expansion of the fruit surfaces during growth was the most important single factor operating against the maintenance of an adequate deposit for the control of chewing insects, particularly the codling moth (*Carpocapsa pomonella* L.). The effect of the growth was particularly great during the early period of development of the fruit, when the surface areas doubled in extent within four or five days. It was reasoned from this work that spray applications would be more effective in maintaining an adequate deposit if the early sprays were applied at more frequent intervals, thus compensating for this rapid increase.

During the season of 1935 ten Stayman Winesap trees in the college orchard were sprayed with a mixture containing lead arsenate 3 pounds, flotation (wettable) sulfur 5 pounds and fish oil 1 quart per 100 gallons of spray mixture. On five of the trees three applications were made at regular intervals of 14 days. On the remaining five trees applications were made at intervals of 4, 10 and 20 days following the first application. Samples of fruit were taken for chemical analyses immediately previous to and following each spray application. More extended measurements of growth rate of fruit of this and other varieties were also taken during this season, from fruit at the tops of the trees as well as from the lower limbs. Study of the results of this experiment led to the following conclusions:

(1) Growth of the fruit at the tops of the trees was considerably more rapid during the greater part of the season than of those at the bottom.

(2) Fruit from the tops of trees of the Stayman Winesap variety increased from 300 square millimeters in area to 950 square millimeters within ten days (between June 1 and June 10). The subsequent increases were progressively less rapid.

(3) In order to provide an adequate deposit on the fruit surfaces during this early period the second cover spray application should follow the first by not more than 5 days. The third application should follow the second by not more than 9 days, while between the third and fourth applications 13 days may elapse. Under conditions of heavy codling moth attack a fifth spray may be necessary, in which case an application 17 days after the fourth should serve to maintain a deposit adequate for protection until early in August.

While the results given above apply only to central Pennsylvania conditions and the details of the method and dates of application may vary with the locality, it is felt that such timing of spray applications is of sufficient importance from an economic and practical point of view to merit consideration by all workers who have to deal with the problem of insect control on materials of this type. The complete results will be published in the near future.

D. E. H. FREAR
H. N. WORTHLEY

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[1] Authorized for publication on January 26, 1937, as Paper No. 760 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

ON THE STRUCTURE OF PECTIN POLYGALACTURONIC ACID

THE fundamental problem of the structure of an oligopolysaccharide consists in elucidation of the places of union of each monose to the other and in the elucidation of the ring structure of each monose. In the special case of the pectin polygalacturonic acid, this information was lacking. We have now succeeded in demonstrating that carbon atoms 4 and 5 are engaged in the ring formation and in the condensation of each unit with its neighboring unit.

This information was obtained by degradation of the polygalacturonic acid with periodic acid which resulted in the formation of levotartaric acid which has been identified as its acid potassium salt.

For $C_4H_5O_6K$, K = 20.78 per cent.; Found K = 20.70 per cent.

$$[\alpha]_D^{25} = -22.0° \text{ (in water)}$$

and for the undissociated acid,

$$[\alpha]_D^{25} = -15.0°.$$

The special function of the hydroxyl groups of each of the carbon atoms 4 and 5 remains to be established. However, it is safe to predict that the hydroxyl group of carbon atom 4 serves for condensation and that of carbon atom 5 for ring formation.

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Reprinted from PLANT PHYSIOLOGY, 12: 571–599, 1937.

FOLIAR DIAGNOSIS: PRINCIPLES AND PRACTICE[1]

WALTER THOMAS

(WITH SEVEN FIGURES)

Introduction

As a result of the broader view now taken with respect to the problems of soil fertility it is recognized that the conditions required for maximum growth must be sought from the facts of plant physiology as well as from those of soil science. For satisfactory growth a particular soil must satisfy certain conditions with respect to temperature, and to nutrient, water, and air supply to roots, the task of the investigator being to discover the best means of bringing the factors that govern these conditions under control. For this purpose the traditional chemical methods both of plant and of soil analyses have definite limitations; some have been abandoned, others are still in use.

In recent years interest has been renewed in the possibilities of the chemical analysis of the plant as a means of studying the nutrient relationships between the crop and the soil with respect to (1) the physiological requirement of a particular species and (2) the rate of supply of nutrients (**10**), *i.e.*, the supplying power of the soil (**27**). It is well established that the composition of a particular plant species is profoundly influenced by the composition of the soil (**40**). Consequently, in order to determine the influence of the soil on the action of fertilizers by means of analysis of the plant it is necessary to experiment first with plants growing in a relatively homogeneous soil (*vide infra*) using systematically laid out plots differentially fertilized. When this is done, the next step is to ascertain the nature of the variation in the composition of plants of the same species and variety in similarly treated (duplicate) plots, the soils of pairs of which may show all types of variations.

There are many reasons, however, why the analysis of the entire plant is not the best means of approach toward the problem of control under field conditions. Apart altogether from the disadvantages of having to dig up and analyze whole plants, the results obtained by the gross analysis of a mass of heterogeneous organs possessing different functions are not a sufficiently sensitive comparative index in reflecting the responses of the plant to differences in its environment (soil and weather) (**41**). The classical investigations of PIERRE (**34**) pointed to the sensitivity of the leaves to changes in composition resulting from differences in environment. But as long as no care was taken to differentiate between leaves of different ages little progress was made. The presentation of the gross analysis of leaves of all ages of a plant species, frequently seen even in present day literature,

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[1] Authorized for publication as paper no. 478 in the Journal Series of the Pennsylsylvania Agricultural Experiment Station.

INTENTIONAL SECOND EXPOSURE

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FOLIAR DIAGNOSIS: PRINCIPLES AND PRACTICE[1]

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(WITH SEVEN FIGURES)

Introduction

As a result of the broader view now taken with respect to the problems of soil fertility it is recognized that the conditions required for maximum growth must be sought from the facts of plant physiology as well as from those of soil science. For satisfactory growth a particular soil must satisfy certain conditions with respect to temperature, and to nutrient, water, and air supply to roots, the task of the investigator being to discover the best means of bringing the factors that govern these conditions under control. For this purpose the traditional chemical methods both of plant and of soil analyses have definite limitations; some have been abandoned, others are still in use.

In recent years interest has been renewed in the possibilities of the chemical analysis of the plant as a means of studying the nutrient relationships between the crop and the soil with respect to (1) the physiological requirement of a particular species and (2) the rate of supply of nutrients (**10**), *i.e.*, the supplying power of the soil (**27**). It is well established that the composition of a particular plant species is profoundly influenced by the composition of the soil (**40**). Consequently, in order to determine the influence of the soil on the action of fertilizers by means of analysis of the plant it is necessary to experiment first with plants growing in a relatively homogeneous soil (*vide infra*) using systematically laid out plots differentially fertilized. When this is done, the next step is to ascertain the nature of the variation in the composition of plants of the same species and variety in similarly treated (duplicate) plots, the soils of pairs of which may show all types of variations.

There are many reasons, however, why the analysis of the entire plant is not the best means of approach toward the problem of control under field conditions. Apart altogether from the disadvantages of having to dig up and analyze whole plants, the results obtained by the gross analysis of a mass of heterogeneous organs possessing different functions are not a sufficiently sensitive comparative index in reflecting the responses of the plant to differences in its environment (soil and weather) (**41**). The classical investigations of PIERRE (**34**) pointed to the sensitivity of the leaves to changes in composition resulting from differences in environment. But as long as no care was taken to differentiate between leaves of different ages little progress was made. The presentation of the gross analysis of leaves of all ages of a plant species, frequently seen even in present day literature, is of no service in diagnosis because neither the relative nor absolute amounts of the mineral entities are the same in leaves of different ages. Moreover, samples taken only at one period can give no information as to the course or nature of the changes in nutrition with growth in relation to differences in the medium. The further development of the method of leaf analysis ("diagnostic foliaire") as a means of studying the course of the absorption of mineral elements under the influence of different growth factors, especially those of fertilizer additions, is largely due to the labors of LAGATU and MAUME.

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Principles of the method of foliar diagnosis

Of the numerous methods employed to control the nutrition of plants under the conditions of practical agriculture only the method of foliar diagnosis has any serious claim to be based on physiological facts established by consistent experimental results obtained over a long period of years (**15, 16, 17, 18, 19, 20, 21, 22, 26**). The laws governing the facts are still obscure, although glimpses of their working have been revealed. Some of the facts and their implications have already been discussed in a series of papers by the writer (**38, 39, 40, 41**).

FACTS UPON WHICH THE METHOD IS BASED

The experimental facts to which the method of foliar diagnosis is linked may be briefly summarized:

1. Two morphologically homologous leaves of the same species and variety are the seat of identical physiological processes when the medium (soil and meteorological factors) of the two is identical and, conversely, are the seat of physiological processes that are different when the medium is different (**13, 18, 19, 21**).

2. The composition of leaves of the same physiological (metabolic) age of plants grown on the same homogenous medium (soil and climatic) and receiving different fertilizer treatments reflects these differences in the sense that whenever a fertilizer element (whether N, P, or K) is effective, as determined by the response of the plant to that element, that response is always associated with an increase of that element in the dry foliage (**18, 19, 37**). This is not, as might appear at first, a principle *a priori*, because neither the processes of migration of the elements into the leaf from the stem nor export from the leaf into the stem is under consideration in the method of foliar diagnosis (*vide infra*), but only the amount of the element present in the leaf at the moment of sampling.

3. The composition—as determined by periodic analysis—of leaves of the same physiological (metabolic) age taken from plants of the same species growing on the same homogenous medium (soil and climatic) but receiving different fertilizer treatments are related to their development (**9, 18, 42**).

4. The magnitude of the variations in the composition, with respect to the dominant nutritive elements, of leaves of the same physiological age from plants of the same species grown in a homogenous soil and subjected either (1) to differential fertilizer treatment under the same meteorological conditions, or (2) to the resultant of meteorological factors acting in each particular growing season, is relatively quite large and, therefore, easily determinable. This delicate sensitivity of the leaf is no doubt related to its rôle as the laboratory of synthesis of the plant (**41**).

AN "IDEAL" SOIL NON-EXISTENT

Reference has been made to a "homogenous" (uniform) soil. Such an ideal soil, of course, does not exist in nature. If an area of land is divided into two plots, A and B, which are managed and fertilized alike and are sown at the same time with selected uniform seed, the yields may be different even when the land appears uniform to the eye. The yields may and, generally, will be widely different if the contour of one part differs greatly from the contour of the other, because the soil factors associated with differences in depth and texture will be different. If the land is fairly level and the soil uniform, however, the relative percentage-difference in yield $\left(\frac{x-y}{x} \times 100\right)$ of two plots treated alike will generally not exceed 5 to 10 per cent., which limit for the purpose of the type of investigation we are considering in this paper is sufficiently close to discover and elucidate rules or principles.

DEFINITION OF FOLIAR DIAGNOSIS

Foliar diagnosis is characterized (**18**) as (a) the chemical composition of a leaf with respect to the dominant nutritive mineral entities at the instant of sampling, taken from a predetermined and suitable position on the stalk. (b) The foliar diagnosis for any given season (year) then will consist of a sequence of chemical states (composition) as determined under (a) on different dates, *i.e.*, at different periods during the growth season. *The composition is based on the dry matter of the leaf without taking into consideration the weight of the dry material at each sampling or the number of leaves sampled from each plant.*

It is clear, therefore, that no physiological significance can be attributed to the foliar diagnosis of any one fertilizer treatment (plot) considered alone. The method is comparative just as the method of analysis of entire plants is comparative (**41**). *Foliar diagnosis considered independently of all other field data and of all other foliar diagnosis has no physiological significance.*

PROPER FORM TO EXPRESS MINERAL ELEMENT CONTENT.—The criticism by BARTHOLOMEW, WATTS, and JANSSEN (**2**) of the method used by the writer of expressing the mineral content of a leaf on the basis of percentage of the element in the dried material is the result of misunderstanding and a confusion of thought. As JAMES (**12**) has well emphasized, the different ways of expressing the mineral content of plants in studies of mineral nutrition must be considered in relation to suitability. Each case must be considered on its merits. In the present instance, there can be no ambiguity nor question that the dry weight unit is the correct one. Furthermore, it is incorporated in the definition of foliar diagnosis (p. 573).

NOMENCLATURE

In investigations concerned with the mechanism of penetration into and translocation within the plant the logical procedure is to refer to all results in terms of the ions K, Ca, Mg, NO_3, H_2PO_4, etc. In physiological investigations having a direct agronomical application, this method is not the most suitable one. The facts are that in practical agriculture and in the arts dependent upon it, a different mode and only one mode of expression is internationally used for the dominant fertilizer principles or entities. These are N for nitrogen, P_2O_5 for phosphoric acid, and K_2O for potash. The writer has adopted this international nomenclature in this paper. And, as a matter of convenience, reference will frequently be made to the three dominant principles as ''elements'' or ''entities.'' This should cause no confusion if the reader keeps in mind that all results are expressed as N, P_2O_5, and K_2O.

TWO METHODS OF EXPRESSING FOLIAR DIAGNOSIS GRAPHICALLY

The graphic presentation of the results of the periodic analyses of the leaves is important in the method of interpretation and may be done in one of two ways: (1) In terms of the percentage of the respective elements in the dried foliage as the ordinate and with the dates of sampling as the abscissa. (2) If only nitrogen, phosphoric acid, and potash are to be considered, the results may also be expressed graphically in trilinear coordinates.

The advantage of the first-mentioned method is that it possesses the capacity of expressing any number of elements for which analysis has been made and of showing the course of each independently. The second method is limited to three entities, in this case to nitrogen, phosphoric acid, and potash—elements which may properly be correctly considered as a unit of NPK, inasmuch as the content of all three in the dry foliage decreases with the age of the leaf, differing in this respect from Ca and Mg which accumulate with age.

BASIC ASSUMPTION IN FERTILIZER PLOT EXPERIMENTS.—The development and yield of a plant is, as already emphasized (p. 571), not exclusively dependent on nitrogen, phosphorus, and potassium. In drouth years water would be the principal factor influencing yields. This factor and others associated with differences in soil texture will also enter in if the soil of the plots is not uniform. Consequently, there can be no *a priori* right to relate the development and yield to the composition of the leaf in terms of nitrogen, phosphoric acid, and potash. Nevertheless, fertilizer field-plot experiments are based on the assumption that the other factors for the plots to be compared are nearly equal. When they are not, recourse is had to many replicates. The present trend is to plan field plots in such a way that each experiment gives a measure of its own error, although, of course, they can never be exact.

QUANTITATIVE INDEX EXPRESSING EFFECT OF A FERTILIZER ENTITY

It has been a tradition to define the response to different fertilizer treatments in terms of the fertilizer or salts used. This point of reference has been used not only by the practical agriculturists but also by mathematicians (**7**), physiologists (**8**), and by agronomists generally. But when the fertilizer (or nutrient salt) treatment is used as the point of reference it is difficult to ascertain whether the effect is due to the sum of the individual elements (ions) or to an effect proportional to their product or even to a more complicated function of their individual effects (**7, 8, 14**).

If, however, a more rational base of reference be taken, *viz.*, the composition of the plant or better its ''reflect,''' *viz.*, a leaf suitably chosen, the problem is considerably simplified. On this basis we may define without ambiguity the quantitative index expressing the effect of any one fertilizer element at the moment of sampling the leaves from any one plot, as the ratio of the amount of that element contained in the leaves of the plant growing on the plot considered, to the amount of that element in the homologous leaves from a plant growing on a plot receiving no fertilizer, sampled at the same time. Likewise, the quantitative index, expressing the effect of any combination of the elements present in the fertilizer used in any plot at the moment of sampling the leaves, may be defined as the ratio of the sum of the elements considered, contained in the leaves of plants growing on this plot at the moment of sampling, to the sum of these elements in morphologically homologous leaves of plants growing on the unfertilized plot.

CONCEPTS OF QUANTITY AND QUALITY OF NUTRITION

Inherent in the expression of the analyses in terms of the dried foliage are two concepts, *viz.*, that of *quantity* and *quality* of the mineral constituents. The concept of *quantity* or intensity of nutrition of the selected leaves consists of the sum $(N + P_2O_5 + K_2O)$ of each element at the moment of sampling expressed as a percentage of the dried material. The *quality* of nutrition is the ratio of these entities to each other at the moment of sampling.

Composite NPK-unit

Expressing the three dominant fertilizer principles as a unit.—The quantities of nitrogen, phosphoric acid, and potash in the dried foliage are expressed as a unit (**23, 24**) as follows:

Let x be the percentage of N, y the percentage of P_2O_5, and z the percentage of K_2O in the dry material of the leaf at the moment of sampling.

Let

$$s = x + y + z. \tag{1}$$

Then s denotes a quantity of mineral nutrients supplied by N, P_2O_5, and K_2O in proportion to the amounts x, y, and z present in the leaf considered at the moment of sampling. Dividing by s we get

$$1 = \frac{x}{s} + \frac{y}{s} + \frac{z}{s}. \tag{2}$$

where $\frac{x}{s}$, $\frac{y}{s}$, and $\frac{z}{s}$ represent fractions which indicate the proportion contributed by N, P_2O_5, and K_2O, respectively, to a unit quantity of the three elements. Let $\frac{x}{s}$ be denoted by x_1, $\frac{y}{s}$ by y_1, and $\frac{z}{s}$ by z_1. Substituting in (2), we get

$$x_1 + y_1 + z_1 = 1. \tag{3}$$

The quantity $x_1 + y_1 + z_1$ represents the composition of the leaf in terms of the proportions in which nitrogen, phosphoric acid, and potash are present in unit quantity of the leaf considered at the moment of sampling and, consequently, it is an expression giving the physiological ratios between N, P_2O_5, and K_2O in the synthetic laboratory of the leaf. It should be emphasized that this unit is independent of the total quantity s of N, P_2O_5, and K_2O in the dry material of the leaf. *The quantity "s," therefore, is a magnitude expressing the quantity of nutrition at the moment of sampling the leaf.*

The magnitude denoted by s indicates the number of units of nitrogen, phosphoric acid, and potash contained in 100 parts of dry matter and, consequently, s expresses the *quantity* (intensity) of nutrition with respect to nitrogen, phosphorus, and potassium. At first blush it might not seem justified to combine into one unit quantities as heterogeneous as N, P_2O_5, and K_2O. However, since the chemical reactions involved are not considered in this expression, but only the amounts of these fertilizer principles in the leaf irrespective of their chemical rôle, and inasmuch as the entities N, P_2O_5, and K_2O are those used in practical agriculture, it would be incongruous to express the characteristic *intensity of nutrition* in milligram equivalents.

Although these two magnitudes representing the *quality* and *quantity* of nutrition are abstractly distinct, they are inseparably connected in the physiological processes of the leaf. They must, therefore, be considered simultaneously when relating the nutrition with respect to NPK of a leaf and its physiological effect on the development of the plant.

Diagrammatic illustration of NPK-unit concept.—These concepts may be illustrated in the following manner (**25**).

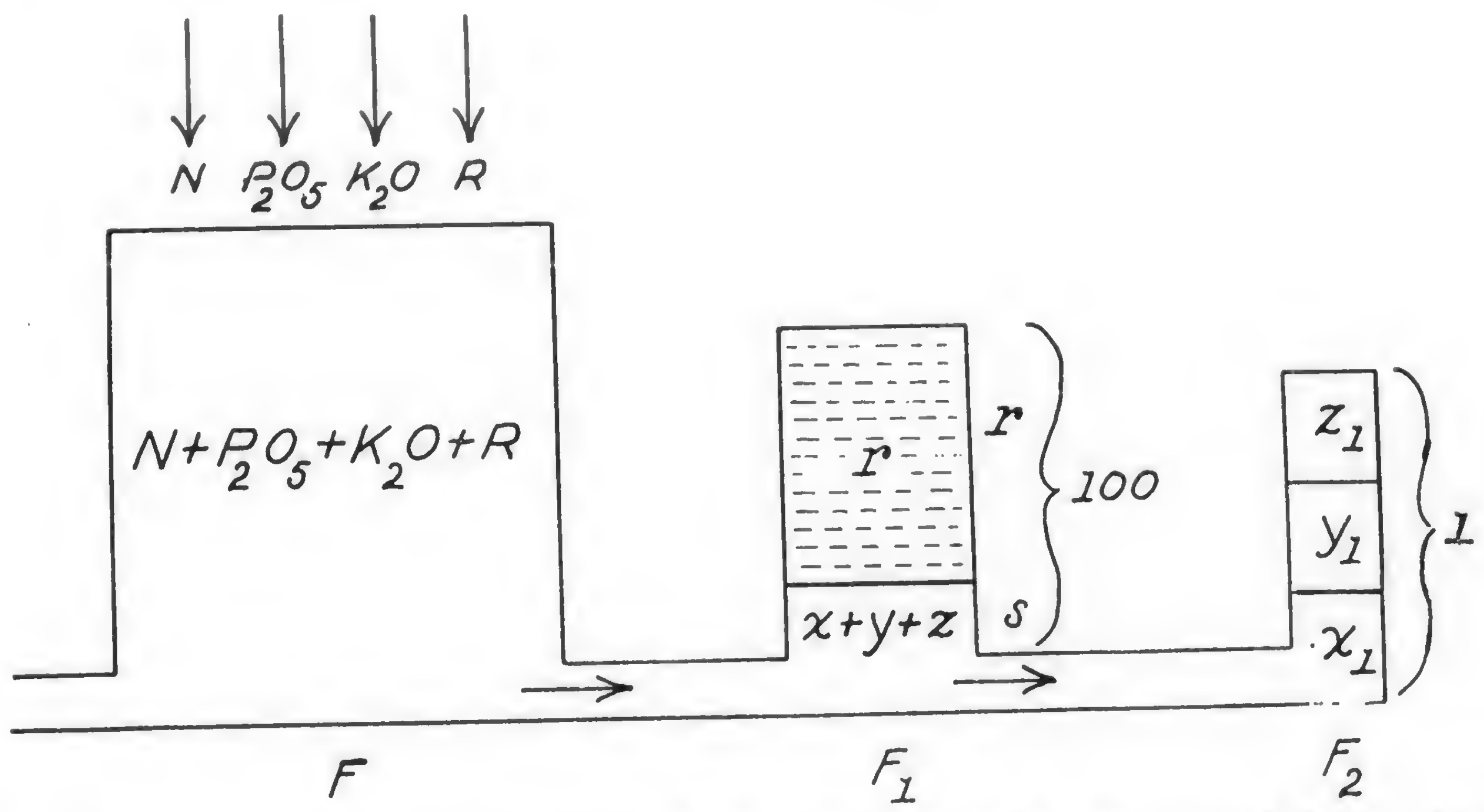

Fig. 1. Diagrammatic representation of the concept of the method of foliar diagnosis.

In figure 1 let *F* represent a leaf into which are migrating substances containing N, P, K plus other materials (R). Of the substances entering the leaf a portion will be elaborated, and a portion will remain in an unelaborated form. Let the quantities of all nutrients present at the particular instant of sampling a leaf be represented by a communicating vessel F_1 of capacity 100, of which $x + y + z = s$ represents the quantities of $N + P_2O_5 + K_2O$ in 100 parts of the dried foliage and r the other substances present also in percentage of the dried foliage. F_1 represents the type of investigation with which foliar diagnosis is concerned. F_1 may then be regarded as an indicator of s.

Let us now imagine another vessel (F_2) *of unit capacity* to be connected with F_1, completely filled at the instant considered, and into which only N, P_2O_5, and K_2O are entering and in the same proportion as they are present in *F* and, consequently, also in F_1. Let us also suppose that N, P_2O_5, and K_2O are separated in F_2 into three layers as shown in the diagram. Then the sum of the quantities x_1, y_1, z_1 of nitrogen, phosphoric acid, and potash, respectively, of F_2 is always equal to 1. The vessel F_2 may then be regarded as the indicator of x_1, y_1, z_1, where $x_1 = \frac{x}{s}$, $y_1 = \frac{y}{s}$, and $z_1 = \frac{z}{s}$.

Calculation of NPK-unit in terms of milligram equivalents.—The *quality* factor of nutrition, *i.e.*, the proportion (ratio) of elements found in the leaf at the respective sampling dates, is most suitably expressed as milligram equivalents (M.E.), because this factor—quality—must be dependent upon and related to the chemical reactions involved. These are calculated as follows:

Let M_x be the percentage of N, M_y the percentage of P_2O_5, and M_z the percentage of K_2O in the leaf at the moment of sampling. Then, reducing to milligram equivalents:

$$E_x = 1000\frac{M_x}{N}; \quad E_y = 1000\frac{M_y}{\frac{1}{6}(P_2O_5)}; \quad E_z = 1000\frac{M_z}{\frac{1}{2}(K_2O)}. \tag{4}$$

Let

$$S = E_x + E_y + E_z.$$

Dividing by S, we get

$$1 = \frac{E_x}{S} + \frac{E_y}{S} + \frac{E_z}{S}. \tag{5}$$

Then $\frac{E_x}{S}, \frac{E_y}{S}, \frac{E_z}{S}$, respectively, represents a fraction which indicates in milligram equivalents the proportional parts of N, P_2O_5, K_2O in a unit quantity of the three elements.

$$\begin{array}{lllll} \text{Let } \frac{E_x}{S} & \text{be denoted by} & x^1, & \text{then} & E_x = x^1S \\ \text{''} \quad \frac{E_y}{S} & \text{''} \quad \text{''} \quad \text{''} & y^1, & \text{''} & E_y = y^1S \\ \text{''} \quad \frac{E_z}{S} & \text{''} \quad \text{''} \quad \text{''} & z^1, & \text{''} & E_z = z^1S \end{array}$$

$$x^1 + y^1 + z^1 = 1. \tag{6}$$

The quantity $x^1 + y^1 + z^1$ represents the composition of the NPK-unit of the leaf at the moment of sampling in terms of milligram equivalents.

To avoid fractional quantities it is convenient to multiply the terms of equation (6) by 100. Then

$$100\,x^1 + 100\,y^1 + 100\,z^1 = 100.$$

Let

$$X = 100\,x^1, \quad Y = 100\,y^1, \quad Z = 100\,z^1.$$

Then

$$X + Y + Z = 100. \tag{7}$$

$X + Y + Z$ is denoted the composite NPK-unit or, for simplicity, the NPK-unit. For each simultaneous value of X, Y, Z there is one point and only one on an equilateral triangle each of whose sides = 100.

Reading the trilinear coordinate graphs.—The higher the locus of a coordinate point moves in the direction of the summit of the triangle representing N = 100 per cent. in the *NPK-unit*, the greater is the proportion of nitrogen in this unit. Likewise, the movement of the locus of a coordinate point toward the left base-apex of the triangle representing $K_2O = 100$ per cent. in the *NPK-unit*, the greater the proportion of potash in this unit. Similarly, changes in the direction of the locus of a point towards the right base-apex of the triangle shows that P_2O_5 in the *NPK-unit* has increased.

Application of method

Plots with treatments used

The potato plants used in this investigation were grown on tier 1, section B, of the Vegetable Fertility Plots of the Department of Horticulture. These plots were laid out in 1916. The description of the experimental lay-out given by Mack (**29**) is in part as follows:

> "The area is laid out in four sections, 245.8 feet wide and 308.4 feet long. . . . Each of the sections is divided into six tiers. . . . The tiers are further divided into seventeen plots, the dimensions of which are 12 by 36.3 feet, and which are separated from each other by guard strips 6 feet wide. The area of the plots, exclusive of guard strips, is exactly 1/100 of an acre."

The four-year rotation consists of cabbage, potatoes, tomatoes, and sweet corn with timothy and rye as a green-manure crop.

The plots examined in this paper together with their treatments and yields (in 1935) are given in table I.

TABLE I

Plots with numbers and treatments

Tier	Plot no.	Commercial fertilizer applied	Commercial fertilizer applied to plot	N, P_2O_5, and K_2O equivalent	Symbol used	Yields per plot
			lb.	*lb.*		*lb.*
1	2	Sodium nitrate	4.0	0.6	N	109
1	3	Superphosphate	6.25	1.0	P	114
1	4	Potassium chloride	1.666	0.8	K	155
1	6	Sodium nitrate Superphosphate	4.0 6.25	0.6 1.0	NP	124
1	7	Sodium nitrate Potassium chloride	4.0 1.666	0.6 0.8	NK	163
1	8	Superphosphate Potassium chloride	6.25 1.666	1.0 0.8	PK	148
1	10	Sodium nitrate Superphosphate Potassium chloride	4.0 6.25 1.666	0.6 1.0 0.8	NPK	162

The complete fertilizer then has a ratio of $N : P_2O_5 : K_2O$ of 0.6 : 1.0 : 0.8 or 6 : 10 : 8.

The plots of tier 1, section B, examined in this experiment were relatively uniform within the limitations defined on page 573. These plots represent only a few of the fertilizer combinations used in these crop fertility experiments. Other amounts with the same and also with different ratios are applied to the remaining plots. The best treatments reported in this paper, *viz.*, NK and NPK, are not those which have given the highest yields in this long continued experiment. The highest yield obtained with commercial fertilizers has been secured by a complete fertilizer containing the same quantity of nitrogen and potash, but containing 50 per cent. more phosphoric acid.

SELECTION AND SAMPLING OF LEAVES

IDEAL SAMPLE.—The ideal sample at any selected sampling date would consist of leaves of the same rank—(physiological or metabolic age)—on the stalks taken at the same time from a sufficient number of plants in a row along the plot to give a sample representative of the plot. Each successive row would then be sampled in like manner on each of the successive sampling dates. The leaf chosen from a plant would conveniently be the one immediately above the caducous leaves at the base of a stalk.

SAMPLING IN RELATION TO SENESCENCE.—Under practical field conditions one must determine beforehand the rank of the leaf or leaves to be selected, for, as explained earlier in this paper (pp. 573, 574), the method consists essentially of a comparison of the mode of nutrition of leaves of the same metabolic or physiological age from plants growing on plots subjected to different factors. To this end the longer the period of observation the greater will be the information obtained with respect to the differences in the course (nature) of the nutrition in response to the different treatments. If two plots receiving different treatments are planted the same day, the plants growing thereon will not reach maturity at the same time. At any intermediate date the plants considered will be at different stages of their life cycle, which can be determined in a general way by observation. With the qualification to be made later, the shorter-lived plants will show the greater amount of color change—from green to yellow and then brown. In the case of the potato leaf a stage is reached at which a golden yellow appears, followed by a further and final color change when it shrivels and becomes a dark brown and shortly after which it drops from the stem. These color changes are in general an indication of their metabolic age and the proportion of yellow and withered leaves on the plant is an index of condition.

CAUSES OF SENESCENCE.—The aging of leaves has been associated with the disappearance of potassium (**3**, **5**), of nitrogen (**4**), of water (**6**), and of all three elements, N. P, and K, simultaneously (**36**). The fact that an increased supply of K has been found to delay breakdown of the mechanism causing aging (**11**) and that at higher concentrations this balance is under certain conditions upset, when yellowing becomes as fast or faster than with a deficiency (**11**), indicates that the mechanism is conditioned by physiological balance. In many instances, cause cannot be distinguished from effect (**3**).

METHOD OF SAMPLING.—Only under the practical field conditions of potato growing, where plants are planted in rows relatively close together and are well nourished, is the problem of sampling in relation to senescence of much moment. In such plants leaves higher up than the second and third may commence to wither before the end of the alloted sampling dates. This difficulty may be overcome in one of two ways. The one would be to take samples of the second or (and) third leaf from a stalk at short intervals of time. Then when the plants are sufficiently developed to take *simultaneously* samples of higher rank on the stalk, *e.g.*, the seventh and eighth leaf, and finally, as the plant matures, leaves still higher, *e.g.*, the twelfth or thirteenth leaf. This method of sampling has the advantage of giving three sets of overlapping samples which, if the plants are not subjected to too violent external disturbances, may be regarded as of corresponding successive physiological ages. The three sets of samples must then be treated separately in reporting results. This method will be illustrated in a later paper.

The second method of overcoming the difficulty is to sample a leaf from a particular position higher on the stalk, *e.g.*, the fourth or fifth leaf, which would give reasonable assurance of enabling observations to be made, say at three or four dates at intervals of about 12 to 20 days. The exact interval between samplings is not important. In the case of the potato plant the writer has found that there is at any given intermediate stage of maturity a progressive increase in nitrogen and phosphoric acid and a progressive decrease in potash of the dry matter of the leaf as one proceeds from the base upward, *i.e.*, the younger the leaf is. (*Cf.* MACGILLIVRAY, **28**, JAMES and PENSTON, **13**, and PENSTON, **32**.)

Accordingly, in the latter method of sampling there will be a slight progressive shift in rank on the stalk of the leaf of that rank with time. However, the differences expressed as percentages of the dried foliage with respect to N, P_2O_5, and K_2O of two consecutive leaves on the stalk of all cultivated plants thus far investigated have been found to be relatively insignificant compared with the differences between the composition of leaves of the same or approximately the same physiological age from plots receiving different treatments. This has been found to be true for any two successive leaves of the potato plants in these experiments. Such differences, therefore, for the purpose of this type of inquiry may be neglected.

Diurnal variations exist in the content of nitrogen, phosphoric acid, and potash in the leaves of the potato (**33**). The magnitude of these diurnal variations is also insignificant compared with the magnitude of the changes considered in the method of foliar diagnosis. Samples, however, should be taken during the same day.

In the present experiments leaves were sampled on four different dates during July and August, and from consecutive rows on each successive date from two stems issuing from the mother tuber from each of the fourteen plants in a row. The number of samplings is arbitrary but at least three samplings should be made. The number of leaves sampled is also immaterial but, obviously, should not vary too greatly from one plot to another. The development of the plants sampled from each plot should be regular and homogeneous, for unless this is the case there would be no justification for considering the samples as representing an average sample.

It should now be clear that foliar diagnosis must be expressed in terms of the rank of the leaf on the stem, the variety of plant, and the year in which samples were taken.

Removal of spray residues, soil, and dust from leaves

The spray materials used contained calcium (and magnesium). It is desirable to remove as much spray material from the leaves as possible, including any soil and dust adhering thereto, so as not to affect the weight of the dry material taken for analysis. The soil and dust are easily removed and most of the spray materials also by brushing with a stiff brush in the field immediately after the samples are taken.

Method of drying leaves

The leaves were dried in a drying oven at 100° C. after being brought in from the field and then finely ground in a Wiley mill.

Determination of nitrogen, phosphoric acid, and potash

Nitrogen was determined by the Kjeldahl-Gunning method to include the nitrogen of nitrates (**1**), phosphoric acid by the Richards and Godden's (**35**) modification of the Pemberton-Neumann (**30, 31**) method, and potash by the Lindo-Gladden method (**1**).

Presentation of results

Table II gives the results of the analysis of the fourth and fifth leaves expressed in (a) percentage of N, P_2O_5, and K_2O in the dried foliage, (b) milligram equivalents, and (c) the NPK-unit.

Calculation of loci of NPK-unit

The following illustrates the method of calculating a coordinate point:

TABLE II

Composition of leaves of rank 4 and 5 at periodic intervals in terms of percentage of N, P_2O_5, and K_2O in dried foliage, milligram equivalents, and the NPK-unit

Date of sampling	Dried foliage			Milligram equivalents				Composition of the NPK-unit		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	$E_x + E_y + E_z$	X	Y	Z
	(M_x)	(M_y)	(M_z)	(E_x)	(E_y)	(E_z)	(S)	$\left(100\frac{E_x}{S}\right)$	$\left(100\frac{E_y}{S}\right)$	$\left(100\frac{E_z}{S}\right)$
	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
					N plot (tier 1, no. 2)					
July 7	5.12	.404	3.97	365.568	17.089	84.561	467.218	78.244	3.658	18.098
July 29	4.42	.534	2.54	315.388	22.588	54.102	392.278	80.450	5.759	13.791
Aug. 9	4.15	.472	2.01	296.310	19.966	42.813	359.089	82.517	5.561	11.922
Aug. 24	3.64	.344	1.77	259.896	14.551	37.701	312.148	83.261	4.661	12.078
					P plot (tier 1, no. 3)					
July 7	4.57	.550	2.41	326.298	25.042	51.333	402.673	81.034	6.218	12.747
July 29	3.46	.521	1.64	247.044	22.038	34.932	304.014	81.260	7.250	11.490
Aug. 9	3.24	.520	1.29	231.336	21.996	27.477	280.809	82.383	7.831	9.786
Aug. 24	2.79	.450	1.11	199.206	19.035	26.643	241.884	82.359	7.868	9.773
					K plot (tier 1, no. 4)					
July 7	4.61	.424	6.32	329.154	17.935	134.573	481.662	68.336	3.725	27.939
July 29	3.87	.540	5.22	276.318	22.842	111.122	410.282	67.349	5.567	27.084
Aug. 9	3.39	.464	4.91	242.046	19.627	104.689	366.362	66.068	5.358	28.575
Aug. 24	2.78	.370	5.00	198.492	15.651	106.500	320.643	61.904	4.881	33.215

TABLE II—(*Continued*)

Date of sampling	Dried foliage			Milligram equivalents				Composition of the NPK-unit		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	$E_x + E_y + E_z$	X	Y	Z
	(M_x)	(M_y)	(M_z)	(E_x)	(E_y)	(E_z)	(S)	$\left(100\frac{E_x}{S}\right)$	$\left(100\frac{E_y}{S}\right)$	$\left(100\frac{E_z}{S}\right)$
	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
NP plot (tier 1, no. 6)										
July 7	5.14	.590	2.53	366.996	24.957	53.910	445.863	82.311	5.598	12.091
July 29	3.87	.532	1.52	276.318	22.504	32.355	331.177	83.435	6.794	9.771
Aug. 9	3.71	.486	1.19	264.894	20.558	25.262	310.714	85.253	6.617	8.130
Aug. 24	3.36	.390	1.09	239.904	16.751	23.110	279.765	85.752	5.987	8.261
NK plot (tier 1, no. 7)										
July 7	5.10	.464	6.63	364.140	19.627	141.176	524.943	69.367	3.739	26.894
July 29	4.03	.510	5.55	287.742	21.573	118.300	427.615	67.290	5.045	27.665
Aug. 9	3.78	.452	5.28	269.892	19.119	112.443	401.454	67.229	4.763	28.008
Aug. 24	3.26	.352	5.46	232.764	14.889	116.234	363.887	63.964	4.092	31.941
PK plot (tier 1, no. 8)										
July 7	4.55	.620	6.76	324.870	26.395	143.967	495.232	65.599	5.330	29.071
July 29	3.36	.510	5.32	239.904	21.573	113.337	374.814	64.006	5.755	30.239
Aug. 9	3.08	.490	5.35	219.912	20.980	113.912	354.804	61.981	5.913	32.105
Aug. 24	2.82	.430	5.04	201.348	18.189	107.309	326.846	61.603	5.565	32.832
NPK plot (tier 1, no. 10)										
July 7	4.98	.584	6.59	355.572	24.703	140.346	520.621	68.297	4.744	26.958
July 29	3.88	.509	4.34	277.032	21.531	92.378	390.941	70.863	5.507	23.630
Aug. 9	3.62	.482	4.70	258.468	20.389	100.046	376.903	68.215	5.381	26.405
Aug. 24	3.14	.392	4.35	224.196	16.582	92.612	333.390	67.249	4.973	27.778

The analytical results (table II) for the N plot (no. 2) sampled July 7 are $N = 5.12$ per cent., $P_2O_5 = 0.404$ per cent., and $K_2O = 3.97$ per cent.

The *intensity of nutrition* (s) of the selected leaves for the N plot on July 7 is, therefore,

$$N + P_2O_5 + K_2O = 5.12 + 0.404 + 3.97 = 9.494$$

To evaluate the *NPK-unit* the percentage values are converted (pp. 577, 578) into milligram equivalents (M.E.) of N, P_2O_5, and K_2O as follows:

$$E_x = 1000 \times 0.0714 \times 5.12 \;= 365.568 \text{ M.E. of N}$$
$$E_y = 1000 \times 0.0423 \times 0.404 = \;\; 17.089 \text{ M.E. of } P_2O_5$$
$$E_z = 1000 \times 0.213 \;\;\times 3.97 \;\; = \;\; 84.561 \text{ M.E. of } K_2O$$

Hence

$$S = E_x + E_y + E_z = 365.568 + 17.089 + 84.561 = 467.218$$

and

$$X = 100\frac{E_x}{S} = \frac{100 \times 365.568}{467.218} = 78.244$$
$$Y = 100\frac{E_y}{S} = \frac{100 \times \;\, 17.089}{467.218} = \;\; 3.658$$
$$Z = 100\frac{E_z}{S} = \frac{100 \times \;\, 84.561}{467.218} = 18.098.$$

These values give the three coordinate points required. It should be noted that $78.244 + 3.658 + 18.098 = 100$. *We then have for each simultaneous value of X, Y, Z a representative point and only one upon the surface of an equilateral triangle the sides of which are expressed in 100 units.*

METEOROLOGICAL CONDITIONS DURING 1935 GROWING SEASON

The foliar diagnosis specified in this paper is that for the fourth and fifth leaves for the potato variety Rural Russet for the year 1935. As a matter of record the meteorological conditions (rainfall and mean daily temperature) for the month preceding the sampling and for the intervals between samplings are given in table III.

TABLE III

RECORD OF METEOROLOGICAL CONDITIONS

Date	Rainfall	Mean daily temperature
	in.	°*F.*
June 1–30	3.88	66.5
July 6–29	5.67	73.5
July 30–Aug. 9	1.19	70.8
Aug. 10–24	0.35	71.4

Interpretation and discussion of results

INDICATIONS GIVEN BY FIRST METHOD OF GRAPHIC PRESENTATION BASED ON PERCENTAGES OF ELEMENTS IN DRIED FOLIAGE AS ORDINATE AND DATES OF SAMPLING AS ABSCISSA

When the percentage of an element in the dried foliage at a particular sampling date is plotted as the ordinate and the dates of sampling as the abscissa, as described in the first method (p. 574), the relationships between the composition of the leaves, with increasing age, from plants growing on the respective plots are clearly brought out and are shown in figure 2.

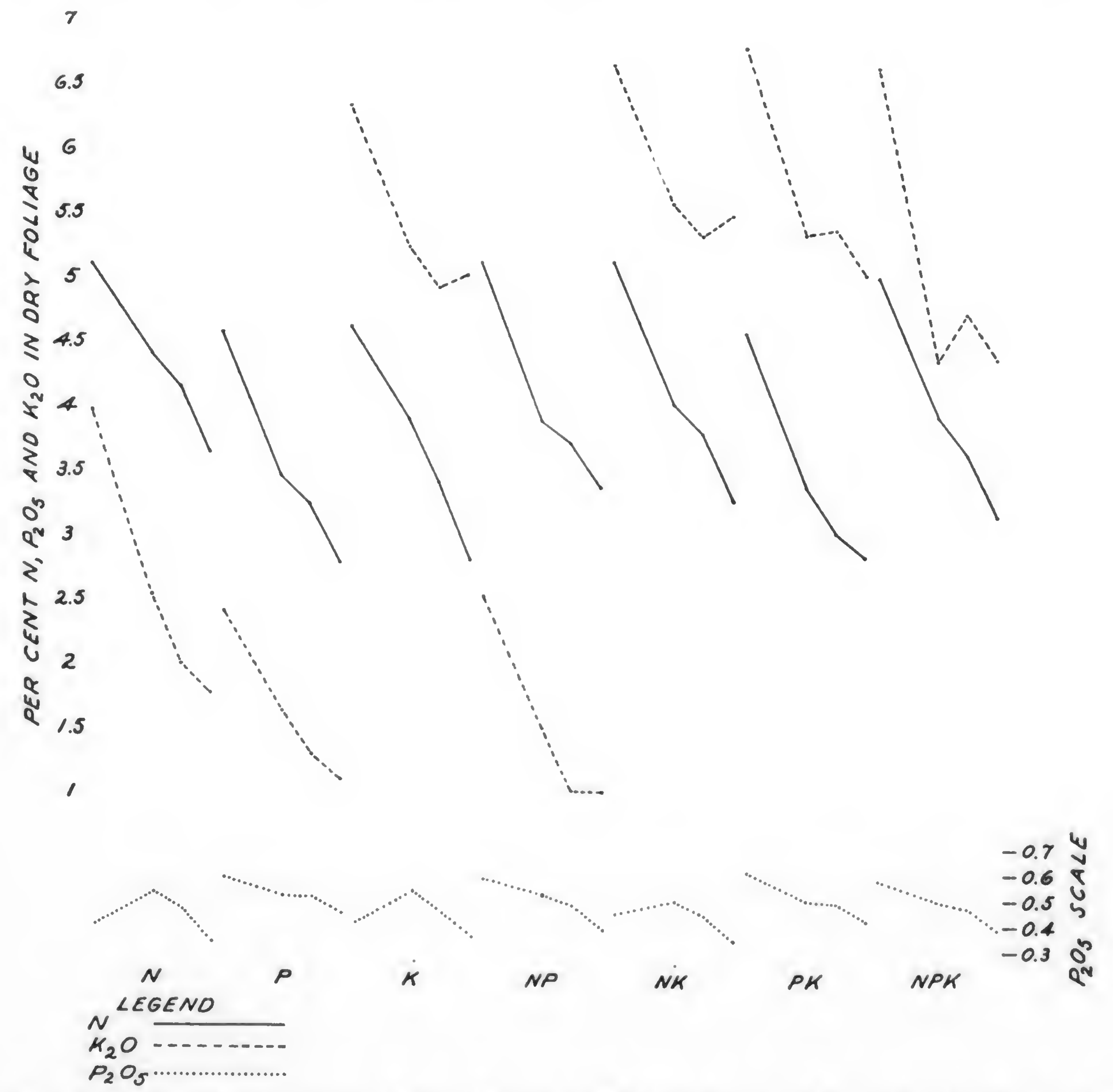

FIG. 2. Periodic analysis of the fourth and fifth leaves of potato plants growing on the differently treated plots showing percentage of elements in the dried foliage as ordinates and the dates of sampling as abscissa.

The indications given by the graphs are summarized below:

NITROGEN GRAPHS.—The content of nitrogen expressed as percentage in the dried foliage of plants which received mineral nitrogen applications is higher throughout the whole period than in those which received none. Furthermore, the nitrogen content decreases with the increasing age of the leaf throughout the whole period in all plots.

PHOSPHORIC ACID GRAPHS.—The content of phosphoric acid expressed as a percentage in the dried foliage from plants which received phosphate applications is higher throughout the whole period than in those which received none. The phosphoric acid content decreases with the increasing age of the leaves of plants growing on plots to which mineral phosphate was added; but in the leaves of all plants which did not receive mineral phosphate additions a maximum occurs at the second sampling (July 29), after which the content of P_2O_5 again decreases regularly with the increasing age of the leaf. This accumulation is undoubtedly a temperature effect. The curve of mean daily temperature is an ascending one up to July 24, after which it begins to descend. This temperature effect would, however, be masked in the plots where phosphorus is rapidly utilized.

POTASH GRAPHS.—The content of potash expressed as a percentage in the dried foliage of all plants which received mineral potash applications is very much higher than in those which did not. The potash content decreases regularly throughout the whole period with increasing age of the leaves of plants growing on plots which did not receive mineral potash. But accumulation has occurred in the leaves from all plants growing on plots which received mineral potash applications. This accumulation commenced in some cases (NPK, no. 10; PK, no. 8) between July 29 and August 9 and in others (NK, no. 7; K, no. 4) between August 9 and August 23.

JAMES (**12**) concludes that in the potato plant potassium is eventually heaped up considerably in excess of the amounts which are necessary to maintain the declining rate of growth. The data presented above show that generalizations with respect to accumulation of potassium are not possible. This accumulation is the result of a lack of physiological balance. It will be shown in a later paper that this accumulation of potassium has not occurred in the leaves of plants growing on plots which received sufficient nitrogen and phosphoric acid for the utilization of the potash.

It is thus seen that periodic analysis of leaves of the same physiological age from plants growing on plots treated with different fertilizers reflects these additions. This also holds true for plots on tier 2 and their duplicates on tiers 4 and 5. The graphs show by their position and their form the nature or course of the nutrition with respect to nitrogen, phosphorus, and potassium in the plants growing on the different plots. The steepness (slope) of a graph of a particular element indicates the relative demand in relation to the supply of that element.

Certain other indications furnished by the data given in table II are best brought out by the second method described on pages 576 and 577. In this method nitrogen, phosphoric acid, and potash are evaluated as a unit-NPK and the results are plotted in trilinear coordinates. This method is illustrated in figures 4, 5, 6, and 7.

INDICATIONS GIVEN BY SECOND METHOD OF GRAPHIC PRESENTATION IN WHICH RESULTS ARE EXPRESSED AS NPK-UNITS AND PLOTTED IN TRILINEAR COORDINATES

In interpreting these graphs (figs. 4, 5, 6, 7) it should be kept in mind that a fertilizer may intervene to produce either (1) an increase in the sum ($N + P_2O_5 + K_2O$), *i.e.*, in the *intensity of nutrition*, or (2) a change in the composition of the *NPK-unit*, or (3) a change in both (1) and (2) simultaneously.

The *intensities of nutrition* are shown in figure 3 with the percentage of

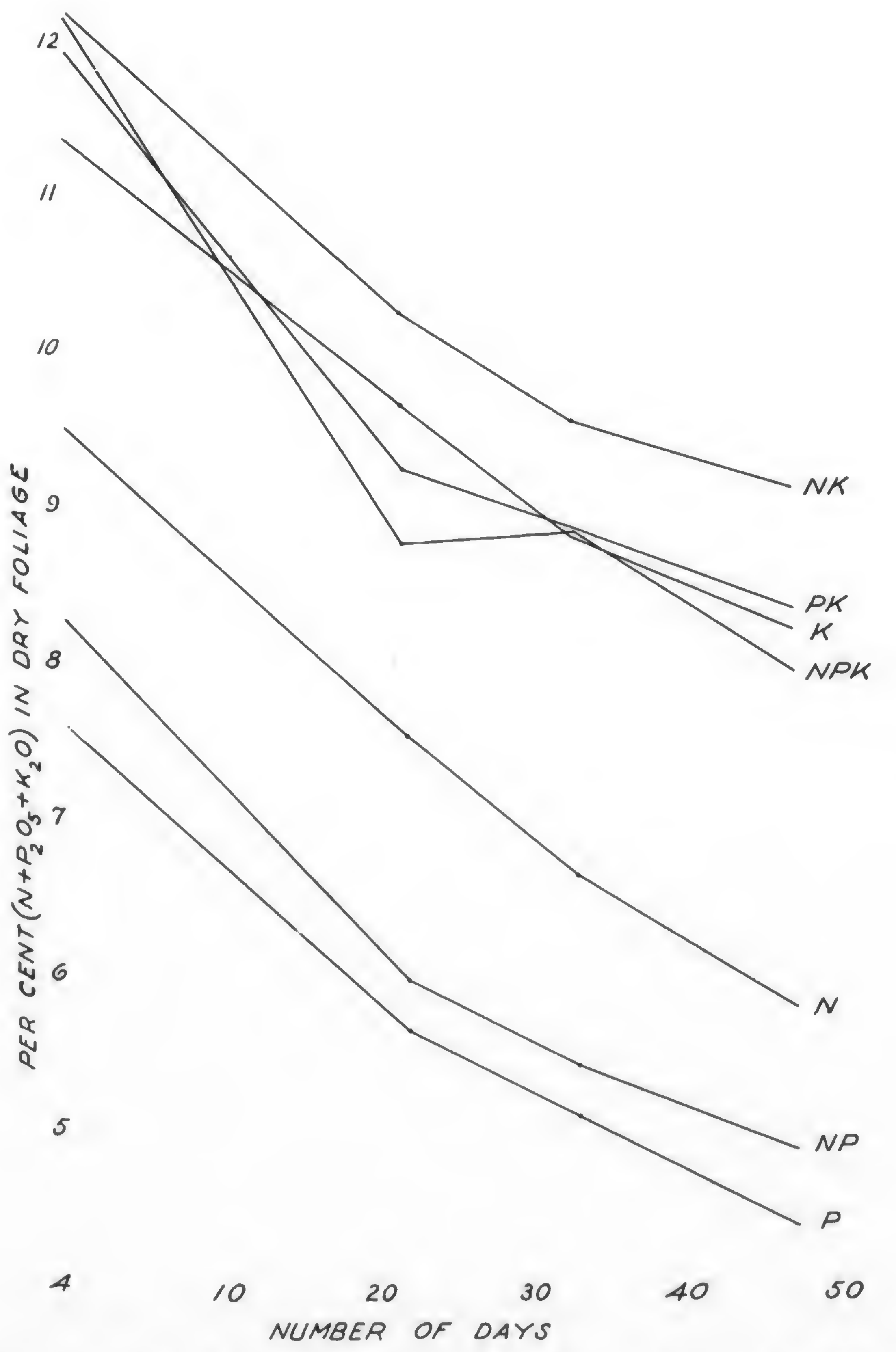

FIG. 3. Relationships of the intensities of nutrition of plants growing on differently treated plots.

TABLE IV

RELATION OF MEAN NPK-UNIT AND MEAN INTENSITY OF NUTRITION AT FOUR SAMPLING DATES TO YIELDS OF POTATOES ON VARIOUS PLOTS

TREATMENT	TIER	PLOT	MEAN NPK-UNITS (MEAN OF 4 SAMPLINGS)					INTENSITY OF NUTRITION (MEAN OF 4 SAMPLINGS)	YIELDS		
			N	:	P_2O_5	:	K_2O		POUNDS PER PLOT	RELATIVE YIELDS NK = 100	CLASS ORDER
			%		%		%		*lb.*	%	
NK	1	7	66.92	:	4.28	:	28.61	10.24	163	100.00	1
NPK	1	10	68.65	:	5.15	:	26.19	9.39	162	99.38	
K	1	4	65.91	:	4.88	:	29.20	9.48	155	95.09	2
PK	1	8	63.29	:	5.64	:	31.06	9.56	148	90.80	
NP	1	6	84.18	:	6.25	:	9.56	6.10	124	76.07	3
P	1	3	81.76	:	7.29	:	10.95	5.65	114	69.99	
N	1	2	81.12	:	4.91	:	13.97	7.34	109	66.93	

$N + P_2O_5 + K_2O$ in the dried foliage from plants growing on the respective plots as the ordinate and as abscissa the dates of sampling. The coordinate points for the respective sampling dates, July 7 and 29, and August 9 and 24, are shown by dots. The displacement in a particular graph from one sampling date to another indicates the changes in the *intensity of nutrition* with the age of the leaves of the plants growing on the plot considered; and the relationship in form and position of the graphs to one another indicates the relative intensities of nutrition (s) resulting from the different treatments.

In figures 4, 5, 6, and 7 are shown in trilinear coordinates the changes in the composition of the *NPK-unit* (*i.e.*, in the *quality of nutrition*) of the selected leaves from plants growing on the differently treated plots. The numbers 1, 2, 3, 4 at the respective coordinate points indicate the successive sampling dates, July 7 and 29, and August 9 and 24, respectively. The displacement of a point from one sampling date to another, therefore, indicates the course of the nutrition with respect to the NPK-unit, as previously defined (p. 578), and which represents the equilibrium between N, P_2O_5, and K_2O at the moment of sampling.

RELATIONSHIP BETWEEN FOLIAR DIAGNOSIS AND YIELDS OF TUBERS.—*The mean NPK-unit.*—Table IV gives (1) the mean NPK-units for each plot, *i.e.*, the mean NPK-unit for the four sampling dates of the selected leaves from plants growing on each plot; (2) the mean intensities of nutrition, and (3) the actual and relative yields of tubers on the basis of the highest yielding of the plots examined being placed at 100.

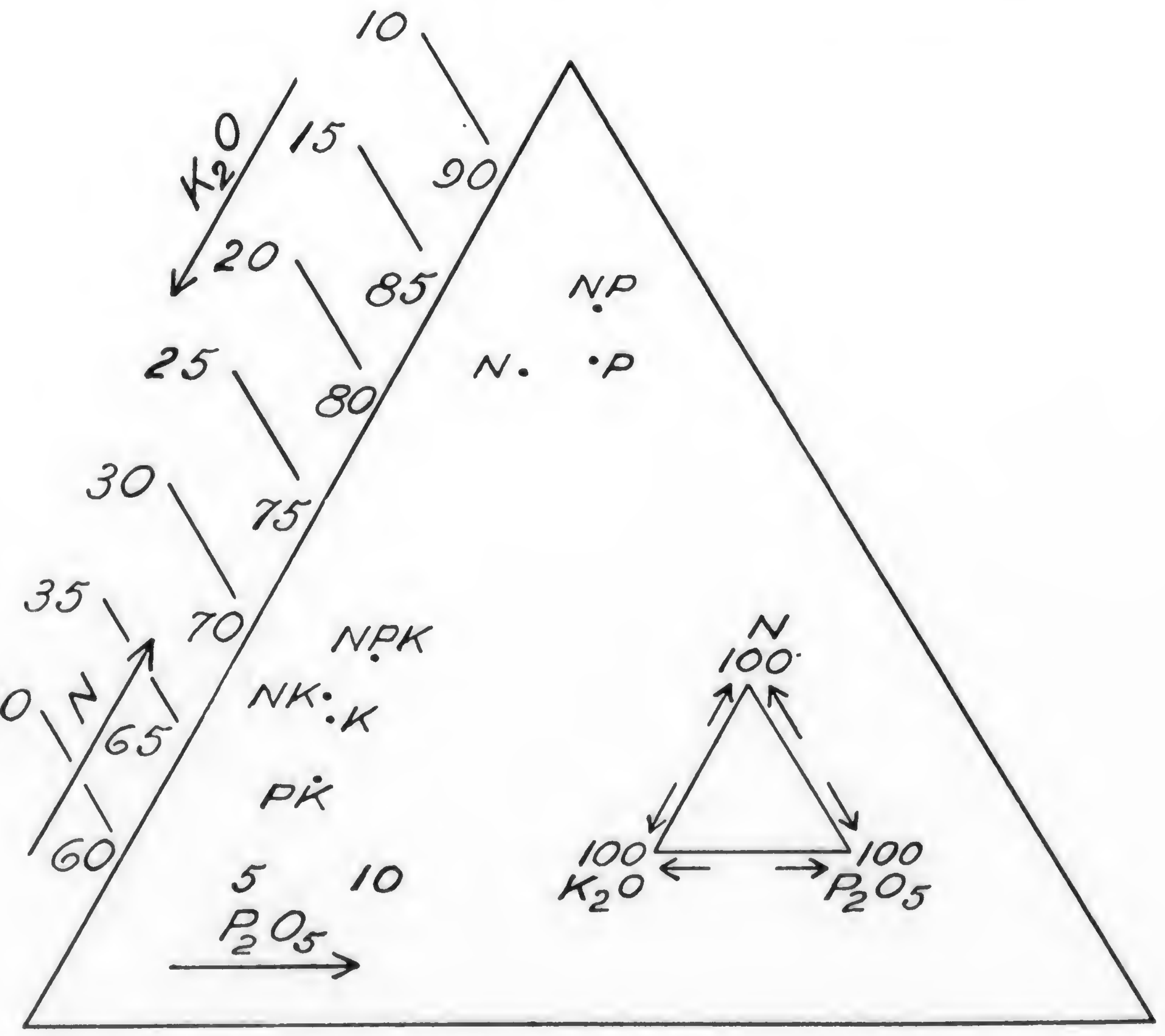

FIG. 4. Relative positions of the mean NPK-units.

The mean NPK-*unit* for each plot is shown graphically in figure 4 by means of a single point for each plot. Each point, therefore, corresponds to the center of gravity (C.G.) of the detailed diagram shown in figures 5, 6, and 7 for the corresponding plot.

The plots are seen to fall into two groups according to their foliar diagnosis, *i.e.*, to their position on the triangle. The one group consisting of the N, P, and NP plots which are found high up toward the N = 100 per cent. apex of the triangle (*i.e.*, the corner representing N = 100 per cent.), and the other group the plots NK, NPK, K, and PK which are found much lower down in the triangle toward the left corner, *i.e.*, the corner representing $K_2O = 100$ per cent.

The latter group can be further subdivided into two groups according to the extent of their displacement toward the base of the triangle. The points representing, respectively, the mean NPK-unit of the plots K (no. 4) and PK (no. 8) are lower than the points representing the plots NK (no. 7) and NPK (no. 10). The groups are designated as follows:

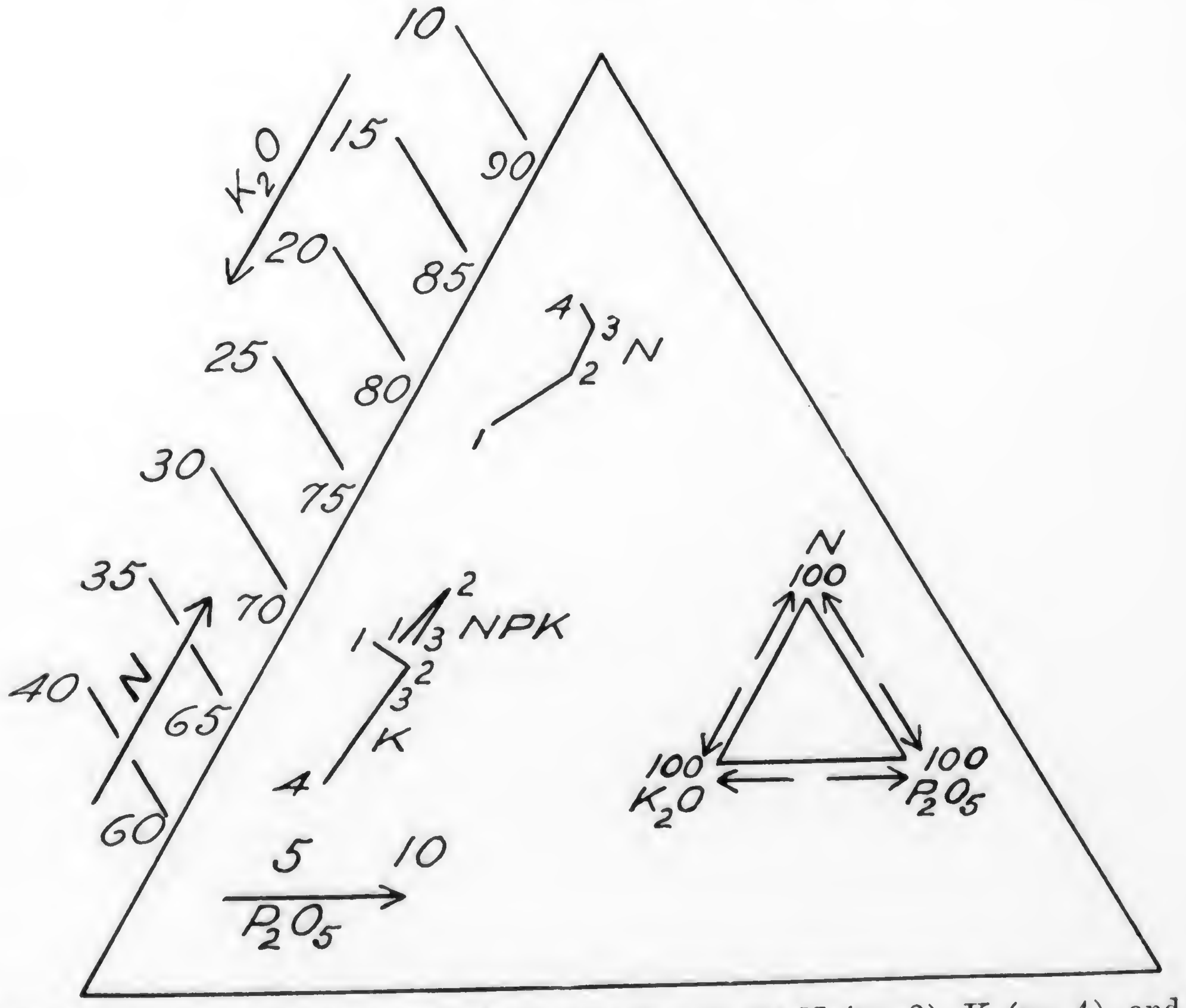

FIG. 5. Course of nutrition of plants growing on the N (no. 2), K (no. 4), and NPK (no. 10) plots as indicated by the changes in their NPK-units from one sampling date to another.

Group 3, NP (no. 6), P (no. 3), N (no. 2), having low yields.
Group 2, K (no. 4), PK (no. 8), having intermediate yields.
Group 1, NK (no. 7), NPK (no. 10), having high yields (the highest reported in this paper).

GRAPHS IN DETAIL.—The displacement of the *NPK-unit* from one sampling date to another for all treatments cannot be shown in one diagram, because of the confusion resulting from the intersection of the graphs from certain of the plots. The detailed graphs showing the course of nutrition are best presented by making use of three diagrams (figs. 5, 6, 7) so arranged that the graphs for the respective treatments are compared with the complete fertilizer plot.

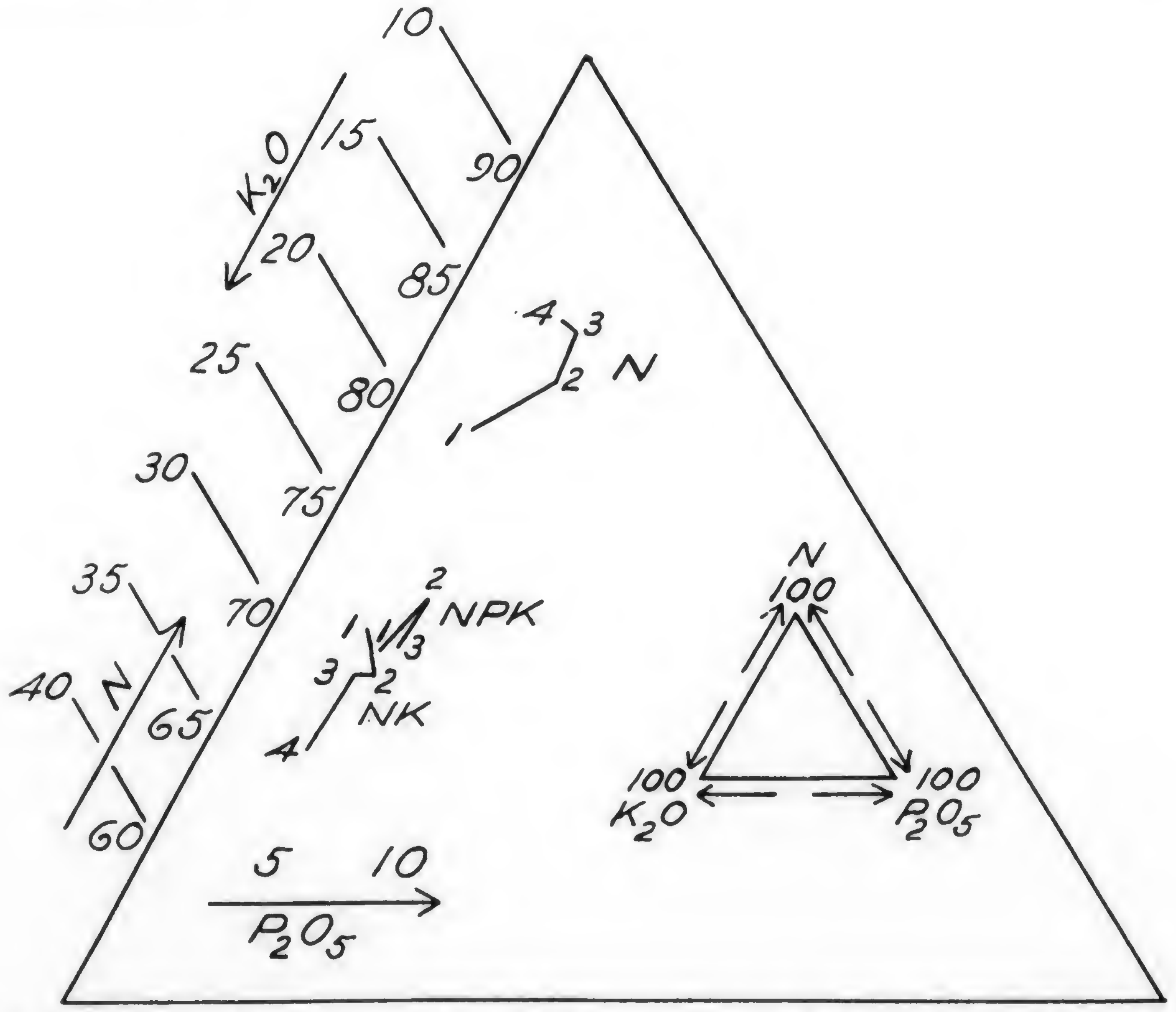

FIG. 6. Course of nutrition of plants growing on the N (no. 2), NK (no. 7), and NPK (no. 10) plots as indicated by the changes in their NPK-units from one sampling date to another.

Foliar diagnosis of low-yielding group 3—N (no. 2), P (no. 3), and NP (no. 6).—The *intensity of nutrition* is very low (range of the mean is 7.34 to 5.65). This low intensity is the result of the very low content of K_2O (see table II, column 3, for plots 2, 3, 6).

Nitrogen in the *NPK-unit* is very high (the range of the mean NPK-unit is 81.1 to 81.7). Potash is very low (the range of the mean NPK-unit is 13.9 to 9.56). Phosphoric acid is high in those which received phosphate additions, *viz.*, NP (no. 6) and P (no. 3).

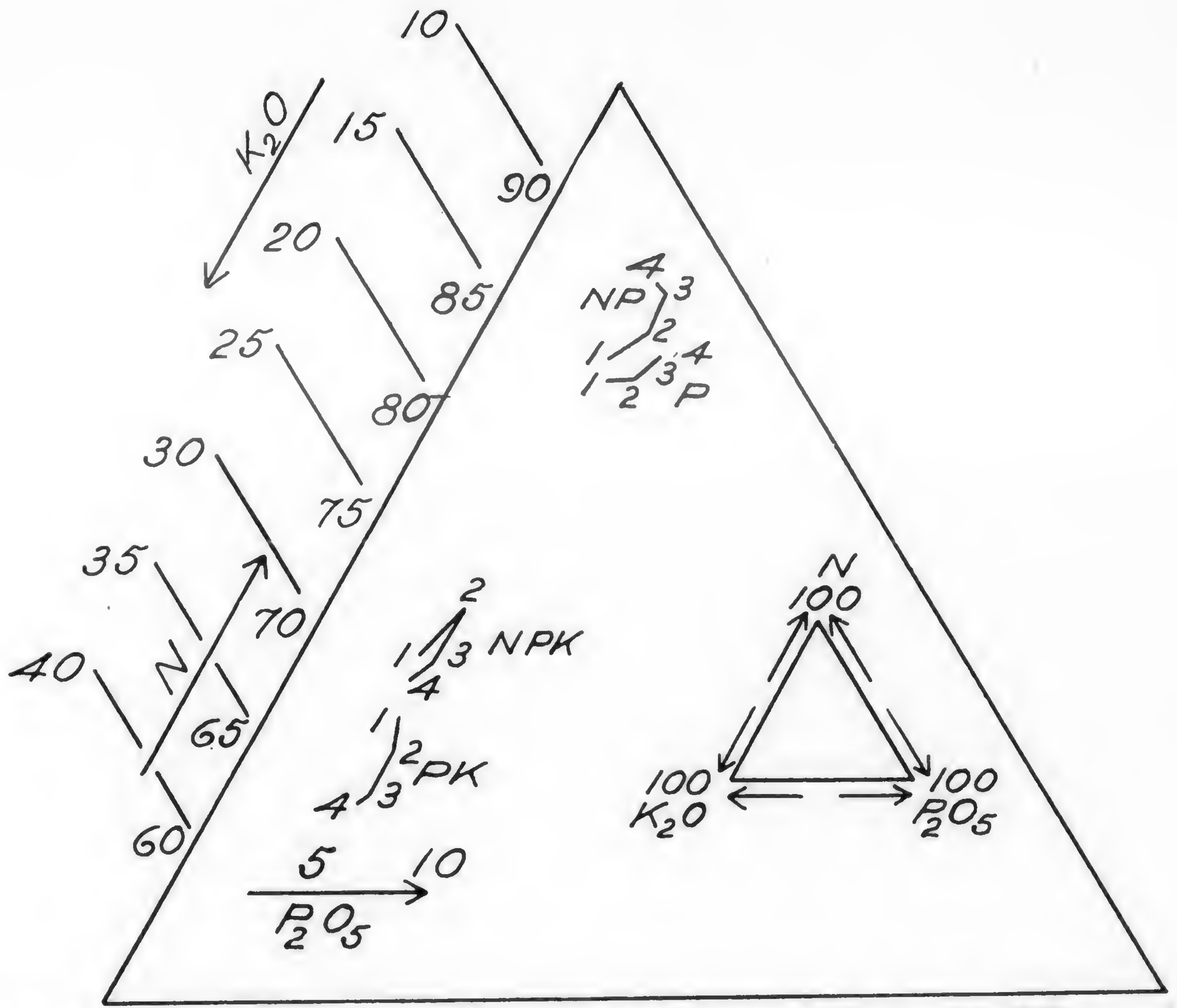

FIG. 7. Course of nutrition of plants growing on the P (no. 3), NP (no. 6), PK (no. 8), and NPK (no. 10) plots as indicated by the changes in their NPK-units from one sampling date to another.

Foliar diagnosis of intermediate-yielding group 2—K (no. 4) and PK (no. 8).—The mean intensity is 9.48 and 9.56, respectively, for the plants growing in the K plot (no. 4) and the PK plot (no. 8). In this group the intensity is slightly higher than that of the complete fertilizer plot (no. 10).

Nitrogen in the *NPK-unit* is much lower than in the low-yielding group 3 and also somewhat lower than in the higher-yielding group 1. The mean NPK-unit is 63.3 and 65.9 in PK (no. 8) and K (no. 4), respectively. Potash in the NPK-unit is higher than in both groups 1 and 3.

Foliar diagnosis of high-yielding group 1—NK (no. 7) and NPK (no. 10).—The mean intensity is 10.24 in NK (no. 7) and 9.39 in NPK (no. 10). The plants growing on plot NK (no. 7) have the highest intensity reported in this paper, but plants growing on three other plots in this tier (not reported in this paper) have still higher intensities. This high intensity of nutrition in NK (no. 7) relative to plants growing on the other plots is due to the phenomenon of the reciprocal effects of the elements (**40**). In this particular case the omission of phosphorus from the complete fertilizer has resulted in a much greater uptake of the elements remaining (compare columns 1, 2, 3 of table II for NK (no. 7) and NPK (no. 10).

Nitrogen in the *NPK-unit* is much lower and potash much higher than in the low-yielding group 3. Phosphoric acid in the NPK-unit has the lowest value in NK (no. 7) of any plot.

Course (nature) of nutrition with respect to NPK-unit in group 3—N (no. 2), P (no. 3), and NP (no. 6).—The course of the nutrition (*mode d'alimentation*) in this low-yielding group is strongly contrasted. This group, however, has one characteristic in common, *viz.*, nitrogen in the *NPK-unit* continuously increases at the expense of the potash with the increasing age of the leaves, as shown by the upward displacement of the graphs at each successive sampling period. In N (no. 2) and NP (no. 6) this tendency is quite marked. Consequently, the application of nitrogen without potash has had a strong tendency to reverse the direction of the nitrogen nutrition which the medium (soil) has tended to impose. This tendency is also observed but to a smaller extent with phosphate applied alone (P plot, no. 3), *i.e.*, without mineral nitrogen applications.

It is also to be noted that in the leaves of plants growing on the N plot (no. 2) and which, therefore, has received no phosphate additions for nearly 20 years other than that added in the system of green manuring, the phosphorus in the *NPK-unit* increases with the age of the leaf up to the third sampling, after which a decrease occurs. The proportion of phosphorus in the *NPK-unit* of the N (no. 2) plot is not, however, as high as that in the leaves from plants growing on the NP (no. 6) or P (no. 3) plots which received mineral phosphates. The continual accumulation of phosphorus throughout the whole period in the NPK-*unit* of plants growing on Plot P (no. 3) is very marked, signifying poor assimilation of this entity (element) by the plants growing on this plot.

Course of nutrition with respect to NPK-unit in group 2—K (no. 4) and PK (no. 8).—The graphs indicate that the course of the nutrition with respect to the *NPK-unit* of the plants growing on these plots is very different throughout the whole period. The relative yields of tubers from plot K (no. 4) to that of plot PK (no. 8) on this tier 1 is 95.1 : 90.8, associated with which is the fact that nitrogen in the *NPK-unit* of plants growing on plot K (no. 4) is higher than are those of plot PK (no. 8) throughout the whole period. The omission of phosphorus, therefore, has on this particular tier resulted in increasing the nitrogen in the *NPK-unit* of plants growing on plot K (no. 4) from one (PK, no. 8) classed as too low in nitrogen. This is another example of the phenomenon of reciprocal effects (**40**).

Course of nutrition with respect to NPK-unit in group 1—NK (no. 7) and NPK (no. 10).—The omission of mineral phosphates has in this case also changed the character of the nutrition with respect to the *NPK-unit*. On July 7 the proportion of nitrogen in the *NPK-unit* in the leaves of plants growing on plot NK (no. 7) is greater than in those from the complete fertilizer plot (no. 10). But, whereas in the former (plot no. 7) the nitrogen in the *NPK-unit* has continuously decreased with the age of the leaf, in the latter (plot no. 10) the nitrogen in the *NPK-unit* has rapidly increased at the expense of the potash during the first period (July 7 to 29) and on July 29 is greater than that of the NK plot. Furthermore, during this first period (July 7 to 29) the phosphorus in the NPK-*unit* of leaves of plants growing on the NK (no. 7) plot has increased much more rapidly than in those from the complete fertilizer plot (no. 10), although phosphorus always remains at a higher level in the NPK-*unit* of plants growing on plot no. 10 throughout the whole period.

The slightly greater increased yields of tubers from NK (no. 7) over those of the complete fertilizer plot (no. 10) in this tier may not be fortuitous. The causative factor may be a higher proportion of potassium in the *NPK-unit* of plants growing on plot NK (no. 7) made at the expense of the nitrogen. In other words, the physiological balance with respect to the dominant nutritive elements in plants growing on plot NK (no. 7) appears to be better than that on plot NPK (no. 10) in this particular tier. Further corroborative evidence is the fact that the *NPK-unit* of NK (no. 7) is nearer the *NPK-unit* of plants growing on the plot giving the highest yield on this tier, *viz.*, manure (plot no. 15).

Effect of omission of an element from complete fertilizer on NPK-unit.—The effects are summarized as follows:

1. The omission of nitrogen from the complete fertilizer has increased the potash and the phosphoric acid in the *NPK-unit* of the leaf throughout the whole period, resulting in decreased yields.
2. The omission of potash from the complete fertilizer has increased the nitrogen and the phosphoric acid in the *NPK-unit* of the leaf throughout the whole period, resulting in decreased yields.
3. The omission of phosphorus from the complete fertilizer has increased the potash in the *NPK-unit* of the leaf at the expense of the nitrogen, resulting in a slight increase of yield.

Effect of omission of two elements from complete fertilizer on NPK-unit.—The effects are summarized as follows:

1. The omission of both phosphorus and potash has increased the nitrogen in the *NPK-unit,* resulting in considerable decrease in yield.
2. The omission of both nitrogen and potash has increased the nitrogen and the phosphorus in the *NPK-unit* with considerable reduction in yields.
3. The omission of both nitrogen and phosphorus has resulted in increasing the potash in the *NPK-unit,* resulting in decreased yields.

We are attempting by means of foliar diagnosis to discover the influence of the soil and the weather on the action of fertilizers. It should be clear, therefore, that no generalization is made from these results with respect to response of potatoes on a Hagerstown clay-loam soil resulting from the treatments used. The foliar diagnosis and the corresponding yields are for the plots indicated on tier 1, section B, of the Vegetable Fertility Plots in the year 1935. In later papers will be reported the relation of foliar diagnosis to optimum nutrition of the potato in the year 1935 and also the relationship of foliar diagnosis to other similarly treated (duplicate) plots in tiers 4 and 5 of this same section B.

Summary

1. The foliar diagnosis of a particular plant species at any given instant of time consists of the mineral composition of a leaf from a definite rank (metabolic age) on the stem. The succession of chemical states with respect to a leaf of the same rank of a particular species as determined at different dates characterizes the foliar diagnosis of that particular plant species (and variety) for the particular growth season examined. The composition is based on the dry material of the leaf without taking into consideration the weight or number of leaves.

2. The physiological facts upon which the method is established are described.

3. Two methods of expressing the results graphically are given and the indications given by each of them presented.

4. It is shown that inherent in the concept of foliar diagnosis is the concept of (1) *quantity* or intensity of nutrition, (2) *quality* of nutrition or the ratio of the dominant elements to one another.

5. The dominant nutritive elements (entities) nitrogen, phosphoric acid, and potash are expressed as a *unit of NPK*. This is designated the *NPK-unit*. The method of deriving the unit is described.

6. Potato plants of the variety Rural Russet growing in 1935 on experimental plots treated with the different combinations of commercial fertilizers were examined by the method. The plots reported in this paper consist of seven on tier 1, section B, of the long-continued Vegetable Fertility plots of the Pennsylvania Agricultural Experiment Station. The treatment consists of the following combinations of fertilizers: N, P, K, NP, NK, PK, NPK.

7. The succession of coordinate points of the *NPK-unit* for the fourth and fifth leaves from the base taken on July 7 and 29, and August 9 and 24, 1935, from plants growing on the differently treated plots are shown in trilinear coordinates. These graphs indicate (1) the course (nature) of the nutrition with respect to the *NPK-unit*, and (2) the relationship between the foliar diagnosis and the yields of tubers.

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THE EFFECT OF BREED CHARACTERISTICS AND THE PLANE OF NUTRITION OF THE COW ON THE VITAMIN A POTENCY OF MILK*

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The natural pigmentation of milk produced by various breeds of dairy cattle under comparable conditions is known to vary with different breeds. The Guernsey and the Jersey breeds produce a more highly pigmented milk than do the cows of the Holstein, Ayrshire, and Brown Swiss breeds. This yellow color is restricted almost entirely to the milk fat and is due primarily to carotene. Since carotene is now recognized as a precursor of vitamin A, it would seem logical to postulate that butter made from Guernsey or Jersey milk should possess greater vitamin A potency than butter made from the milk of the Holstein, the Ayrshire, or the Brown Swiss breeds. Furthermore, Jersey and Guernsey milks, in addition to having a higher color value, also have a higher average fat content. In view of these facts, it would appear that there should be a difference in the vitamin A potency of these milks, especially if carotene alone is responsible for this biological activity. Milk fat, however, does not owe all of its vitamin A activity to carotene. Morton and Heilbron (1) have demonstrated by spectographic analysis that milk fat contains both carotene and vitamin A. Moore (2) has likewise demonstrated that milk fat contains both carotene and vitamin A and that cows convert carotene into vitamin A.

Davis and Hathaway (3) studied the vitamin A potency of milk from Holstein, Jersey, Ayrshire, and Guernsey cows, and concluded that there was no significant difference in the vitamin A content of these milks. Hathaway and Davis (4) reported that Holstein cream contained more vitamin A than Jersey cream. Wilbur, Hilton and Hauge (5) made a comparison of the vitamin A activity of butters from one group of Ayrshire and two groups of Guernsey cows fed under identical conditions. As the result of these studies they concluded that the vitamin A activity of the samples of butter were similar, regardless of the breed of cows or the color of butter. Sutton and Krauss (6) of the Ohio Station concluded that Hol-

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stein milk fat had a greater vitamin A potency per unit weight than did Guernsey milk fat, in spite of the fact that the Guernsey milk fat was richer in carotene. Booth, Kon and Gillam (7) compared the vitamin A activity of Shorthorn and Guernsey butters produced from cows kept under comparable conditions of feeding and management. These butters were found to be identical in vitamin A activity. Baumann *et al.* (8) made a study of the influence of breed and diet of cows on carotene and vitamin A content of the butter produced. These investigators used spectroscopic methods for determining carotene and vitamin A, and reported definite breed differences when the cows received high and low carotene diets. However, when total biological activity of the several butters were calculated, they found this breed difference to be small.

It is clearly evident that the data presented in the foregoing literature are not in complete agreement with regard to several pertinent points. It is therefore obvious that additional information on a number of these points would be of material value.

The present investigation was undertaken in order to obtain further data concerning the effect of breed characteristics and the plane of nutrition of the cow on the vitamin A potency of milk.

EXPERIMENTAL PROCEDURE

The present investigation may be regarded as consisting of four distinct phases, viz.

I. The vitamin A potency of milks from cows representing breeds that normally produce highly pigmented and poorly pigmented milks when fed a good ration similar to those commonly employed in commercial feeding practice.

II. The vitamin A potency of milks from cows as in I, but fed a pigment poor ration.

III. The vitamin A potency of milks from cows as in II when large daily doses of commercial carotene were included in the ration.

IV. The vitamin A potency of milks produced by five standard dairy breeds under identical feeding conditions.

The cows selected for each phase of this study were true representatives of the respective breeds and the stages of lactation and gestation were as nearly identical as possible. The cows were milked the same number of times daily, were treated as nearly alike as possible and were maintained in individual stalls so constructed that access to food substances other than those intended for the particuar animals was not possible. Each animal received, daily, an accurately weighed quantity of ration in the form of a grain mixture and roughage. An aliquot portion of the milk produced at each of the three milkings during the twenty-four hour period was fed the same day to test rats, in the usual manner, in order to determine its vitamin A potency. This procedure was followed in studying each phase, with minor modifications which will be mentioned later.

PRESENTATION OF DATA

I. *The vitamin A potency of milks from cows representing breeds that normally produce highly pigmented and poorly pigmented milks when fed a good ration similar to those commonly employed in commercial feeding practice*

In the first phase of the investigation an attempt was made to determine the vitamin A content of milks from Guernsey and Holstein cows which were fed a good dairy ration that is commonly fed in commercial feeding practice. For this phase of the study two groups of cows (two Holstein and two Guernseys in each group) were selected from the experiment station herd as experimental subjects.

The four cows in Group I were placed on a diet composed of a good quality dehydrated clover hay and a grain mixture (300 parts corn and cob meal, 200 parts of oat feed, 250 parts barley feed, 50 parts distillers' grain, 125 parts cottonseed meal, and 50 parts gluten feed) which was the usual ration fed to the test cows in the station herd. The cows received this diet for one month previous to the test period. The purpose of this preliminary feeding period was to give the cows sufficient time to start producing milk uniform in vitamin A.

Although the rat data were fairly conclusive as to the vitamin A potency of the milk produced by the four cows, it was deemed advisable to repeat the experiment in order to have more conclusive data. For this purpose other Holsteins and Guernseys were used. These cows received the same basal diet and roughage and were handled in the same manner as the cows in the previous experiment.

Tables I and II present the vitamin A potencies of milks produced by representative Guernsey and Holstein cows while receiving a normal dairy ration.

Table II shows the average vitamin A potencies of milks produced by a second series of Guernsey and Holstein cows receiving a normal dairy ration.

From the data presented in Tables I and II, it is quite evident that equal volumes of milk from cows of different breeds receiving a normal dairy ration are not equally potent in vitamin A as reported by Davis and Hathaway (3). There appears also to be a wide difference in the vitamin A potency of milks produced by different cows of the same breed. The milks from Holstein cows Nos. 1709 and 1711 were very low in vitamin A as compared with the milk from Guernsey cow No. 1673 and Holstein cow No. 1489. The rats receiving 0.6 cc. of milk from cows Nos. 1709 and 1711 were unable to maintain their body weights, while the rats receiving a like amount of milks from cows Nos. 1673 and 1489 made an average gain of 31 and 34

TABLE I

The vitamin A potencies of milks produced by representative Guernsey and Holstein cows receiving a normal dairy ration

COW NO.	BREED	NUMBER OF RATS	AMOUNT MILK FED CC.	AVERAGE WEIGHT OF RATS AT END OF DEPLETION PERIOD GMS.	AVERAGE WEIGHT OF RATS AT END OF EXPERIMENT GMS.	AVERAGE GAIN IN WEIGHT GMS.	AVERAGE DAILY GAIN PER GRAM MILK FED	APPROXIMATE VITAMIN A UNITS PER QUART (SHERMAN)
1453	Holstein	2	0.4	69	66	−3	0.617	1447
1453	Holstein	4	0.6	83	97	14		
1453	Holstein	5	0.8	79	107	28		
1489	Holstein	3	0.4	84	84	0	1.07	3511
1489	Holstein	3	0.6	85	119	34		
1489	Holstein	5	0.8	72	106	34		
1571	Guernsey	2	0.4	78	77	−1	0.902	2620
1571	Guernsey	5	0.6	74	100	26		
1571	Guernsey	5	0.8	79	104	25		
1673	Guernsey	1	0.4	78	88	10	1.24	3203
1673	Guernsey	4	0.6	77	108	31		
1673	Guernsey	5	0.8	78	123	45		

TABLE II

The average vitamin A potencies of milks produced by a second group of Guernsey and Holstein cows receiving a normal dairy ration

COW NO.	BREED	NUMBER OF RATS	AMOUNT MILK FED CC.	AVERAGE WEIGHT OF RATS AT END OF DEPLETION PERIOD GMS.	AVERAGE WEIGHT OF RATS AT END OF EXPERIMENT GMS.	AVERAGE GAIN IN WEIGHT GMS.	AVERAGE DAILY GAIN PER GRAM MILK FED	APPROXIMATE VITAMIN A UNITS PER QUART (SHERMAN)
1709	Holstein	6	0.5	127	124	− 3		
1709	Holstein	2	0.6	141	130	−11		
1711	Holstein	4	0.5	121	105	−16		
1711	Holstein	5	0.6	95	90	− 5		
1546	Guernsey	5	0.5	128	150	22	1.22	1868
1546	Guernsey	4	0.6	120	145	25		
1548	Guernsey	6	0.5	128	128	0		
1548	Guernsey	4	0.6	115	134	19	0.50	1550

grams respectively during the experimental period. Holstein cow No. 1453 and Guernsey cows Nos. 1546 and 1548 produced milks containing about the same amounts of vitamin A. The vitamin A potency per unit volume of these milks was midway between the two extremes mentioned above. Wilber, Hilton and Hauge (5) stated that under similar feeding conditions the higher the percentage of fat in the milk, the greater was its vitamin A potency. The data presented herein are not in complete agreement with the above findings, as some cows produced milk of the same fat content and were found to differ considerably in vitamin A potency. In our study the vitamin A potency of the milk of individual cows was determined, whereas the vitamin A potency of the pooled milk from groups of cows was determined by the above mentioned investigators. If, however, the average vitamin A potency of the milk of the four cows from each breed is considered, the results are in close agreement with those of Wilbur and associates.

II. *The vitamin A potency of milks from cows representing breeds that normally produce pigment rich and pigment poor milks, the ration fed to all cows being of pigment poor quality*

The cows used in the latter part of the former experiment were also used for this phase of this investigation. After receiving a normal dairy ration for two months, these cows were fed a pigment poor ration. This ration consisted of a grain mixture, timothy hay of very poor quality and dried beet pulp. The grain mixture (300 parts bran, 200 parts oats, 200 parts barley, 100 parts linseed meal, 100 parts cottonseed meal, and 9 parts salt) was fed in amounts proportionate to the milk production. Twelve pounds of timothy hay of poor grade were fed to each cow daily. The Holstein cows received eight pounds of dry beet pulp daily, while the Guernseys received but six pounds daily. The cows received this diet from November, 1933, until June, 1934. After seven months, the cows were in poor physical condition, had lost weight, had declined in milk production, and had manifested marked symptoms of diarrhea. Five attempts were made during this seven-month period to determine the vitamin A potency of the milks from each of the four cows. The estimated amount of milk necessary to induce unit gain in weight was calculated at the beginning of each succeeding determination with the aid of the data previously established. These estimations were difficult to make, for at the time the experiment was being carried out no data were available to indicate the rapidity of depletion or the amount of time required to deplete the cows of their body stores of vitamin A. As a result, a number of these estimations proved to be low, and the rats receiving such quantities of milk were unable to maintain their body weights. In the last weeks of the experiment sufficient data had been collected, however, to make it possible to estimate more accurately the volume of milk necessary to induce unit gain. Table III presents a summary of the biological data obtained while testing the milk from these cows which received a pigment-poor ration. During this phase of the work, many of the levels chosen proved to be too low, with the result that many of the rats on such levels died before the end of the curative period. The data from those rats which made significant responses are included in this table. The table contains a summary of the growth responses of only 130 rats, although 250 rats were used in this phase of the study.

The data in Table III show that Guernsey cow No. 1546 produced milk containing the highest vitamin A potency per unit volume. Holstein cow No. 1711, on the other hand, produced milk with the lowest vitamin A potency. It is also evident that the milk from the two Guernsey cows was richer in vitamin A than the milk of the two Holstein cows. The milk from Guernsey cow No. 1546 contained approximately 1550 units per quart at the beginning of the depletion period and 513 units at the end of the depletion period. This reduction in vitamin A represents approximately 66 per cent of the total. Likewise, milk from Guernsey cow No. 1548 contained 1550 units of vitamin A at the beginning of the depletion period and less than 405 units at the end of the seven months' period. This represents a decrease of approximately 73 per cent of the total vitamin A potency.

It is impossible from the data obtained to calculate the vitamin A potency of the milks from Holstein cows Nos. 1709 and 1711 at the beginning of the depletion period. However, it may be assumed that it contained approximately 900 units per quart in the beginning. The data obtained show that they contained an average of 255 units per quart at the end of the depletion period. If the above assumption is correct, the milks from the two Holstein cows lost 70 per cent of their original vitamin A potency during the seven months' period on a pigment-poor diet. This reduction in vitamin A potency is approximately the same as that found with the Guernsey milks. The data indicate that the rate of decrease in total vitamin A potency of milks produced under these adverse feeding conditions was about the same for the two breeds studied.

The foregoing results agree with those reported by Fraps, Copeland and Treichler (10). These investigators reported that the milks which they tested contained from 33 to 38 Sherman units of vitamin A per gram of fat in the beginning and from 5 to 12 units after the cows had been on a pigment-poor diet for five months. Gillam and coworkers (9) in England found that in the case of cows receiving a normal winter ration of hay and concentrates, the vitamin A potency of butter fell from approximately 16 International units of vitamin A per gram in the beginning to approximately 5 units at the end of six months. This represents a reduction of approximately 68 per cent, a result which compares favorably with that obtained in these studies.

These results are in agreement with those reported in Tables I and II

TABLE III

Results obtained in a progressive series of vitamin A assays made on milks produced by representative Guernsey and Holstein cows receiving a pigment poor ration

PERIOD	BREED	COW NO.	NUMBER OF RATS	DOSE CC.	AVERAGE WEIGHT OF RATS AT END OF DEPLETION PERIOD GMS.	AVERAGE WEIGHT OF RATS AT END OF EXPERIMENT GMS.	AVERAGE GAIN IN WEIGHT GMS.	AVERAGE DAILY GAIN PER GRAM MILK FED	APPROXIMATE VITAMIN A UNITS PER QUART (SHERMAN)
11/25 to 12/30	Guernsey	1546	5	0.6	115.2	122.8	7.6	.36	Less than 1550
12/19 to 1/23	Guernsey	1546	4	0.6	150.5	150.5	0.0		
12/19 to 1/23	Guernsey	1546	3	0.8	114.0	131.3	17.3	.62	1167
3/25 to 4/29	Guernsey	1546	3	1.0	118.3	138.0	19.7	.56	934
3/25 to 4/29	Guernsey	1546	3	1.3	104.0	132.6	28.6	.62	710
3/25 to 4/29	Guernsey	1546	4	1.6	117.8	138.7	21.0	.37	551
4/15 to 5/29	Guernsey	1546	5	1.6	67.2	80.0	12.8		
6/ 9 to 6/13	Guernsey	1546	7	1.8	104.4	125.0	20.6	.32	513
11/25 to 12/30	Guernsey	1548	5	0.6	114.6	104.8	9.8		
12/19 to 1/23	Guernsey	1548	4	0.6	108.0	118.5	10.5	.49	1550
3/25 to 4/29	Guernsey	1548	4	1.0	115.7	109.0	- 6.7		
3/25 to 4/29	Guernsey	1548	4	1.3	117.7	121.5	3.7		
3/25 to 4/29	Guernsey	1548	4	1.6	113.2	122.5	9.2		
4/12 to 5/29	Guernsey	1548	5	2.0	73.0	85.4	12.4	.17	467
6/ 9 to 6/13	Guernsey	1548	9	2.3	102.6	108.7	6.1	.08	Less than 405

TABLE III—(*Continued*)

PERIOD	BREED	COW NO.	NUMBER OF RATS	DOSE CC.	AVERAGE WEIGHT OF RATS AT END OF DEPLETION PERIOL GMS.	AVERAGE WEIGHT OF RATS AT END OF EXPERIMENT GMS.	AVERAGE GAIN IN WEIGHT GMS.	AVERAGE DAILY GAIN PER GRAM MILK FED	APPROXIMATE VITAMIN A UNITS PER QUART (SHERMAN)
11/25 to 12/30	Holstein	1709	4	0.8	111.2	111.0	−0.2		900
12/19 to 1/23	Holstein	1709	4	0.8	111.5	100.2	−11.3		
3/25 to 4/29	Holstein	1709	2	1.0	110.0	95.5	−14.5		
3/25 to 4/29	Holstein	1709	4	1.3	122.2	124.5	2.3		
3/25 to 4/29	Holstein	1709	4	1.6	112.5	115.7	3.2		
4/15 to 5/29	Holstein	1709	4	2.0	73.5	75.7	2.2		
6/ 9 to 6/13	Holstein	1709	9	3.5	107.6	124.3	16.7	.13	266
11/25 to 12/30	Holstein	1711	4	0.8	101.7	91.2	−10.5		900
12/19 to 1/23	Holstein	1711	3	0.8	127.6	113.6	−14.0		
12/19 to 1/23	Holstein	1711	3	1.0	100.6	87.3	−13.3		
3/25 to 4/29	Holstein	1711	4	1.0	102.5	81.0	−21.3		
3/25 to 4/29	Holstein	1711	4	1.3	107.7	110.5	2.8		
3/25 to 4/29	Holstein	1711	4	1.6	106.5	106.3	−0.2		
4/15 to 5/29	Holstein	1711	2	2.0	67.0	71.0	4.0		
6/ 9 to 6/13	Holstein	1711	6	3.8	109.0	124.6	15.6	.12	245

of the present study, namely, that Guernsey milk contains more vitamin A per unit volume than Holstein milk.

III. *The vitamin A potency of milks from cows representing breeds that normally produce pigment-rich and pigment-poor milks, the ration fed to all cows being pigment-poor but highly fortified with a commercial carotene preparation*

The cows used in the previous experiment that had been depleted of their body stores of vitamin A were also used for this study. It was planned to feed daily 150,000 A.D.M.A. units of vitamin A in the form of carotene to each of the depleted cows for a period of twelve days. A twelve-day feeding period was subdivided into three equal periods and a biological study for increased vitamin A potency was made on aliquot portions of the milk produced during each of these periods. All the milk from each of the four cows was collected and kept in ice water for four days, after which it was pooled. Approximately 80 cc. portions of the composited milks were placed in individual cardboard containers and these in turn were placed in a cold room at – 15° F. where they were frozen and stored until used. The milks from the last four days of the depletion period were also collected and stored in the same manner. As the milks were needed for the biological assays they were removed from cold storage and allowed to thaw at room temperature, previous to feeding.

The four depleted cows were fed daily carotene equivalent to 150,000 units of vitamin A for a twelve-day period. This carotene was supplied to each animal by feeding 51 cc. of a commercial carotene preparation which contained 3,000 A.D.M.A. units of vitamin A per gram.* This amount of carotene was considered sufficient to cause the cows to regain their body stores of carotene and to secrete milk of at least normal vitamin A potency by the end of the twelve-day period. The twelve days were divided into three periods to study (by the biological response method) any increase in vitamin A potency of the pooled milks during these successive collection periods. The method of sampling and preserving of the milks is described above. Since it was necessary to know the vitamin A potency of the milks before the carotene feeding began, the milk from the last four days of the depletion period was collected and pooled.

TABLE IV

Summarizing the results of a progressive series of vitamin A assays made on milks produced by representative Guernsey and Holstein cows after being changed from a pigment poor ration to a pigment rich ration

CAROTENE FEEDING PERIOD	COW NO.	NUMBER OF RATS	DOSE CC.	AVERAGE WEIGHT OF RATS AT END OF DEPLETION PERIOD GMS.	AVERAGE WEIGHT OF RATS AT END OF EXPERIMENT GMS.	GAIN IN WEIGHT GMS.	APPROXIMATE VITAMIN A UNITS PER QUART (SHERMAN)
Last 4 days of depletion period	1546	7	1.8	104.4	125.0	20.6	450
First 4 days carotene feeding	1546	9	1.8	81.3	101.2	19.9	
Second 4 days carotene feeding	1546	7	1.8	82.0	107.2	25.2	
Third 4 days carotene feeding	1546	6	1.8	98.6	127.5	28.9	
Last 4 days of depletion period	1548	9	2.3	102.6	108.7	6.1	400
First 4 days carotene feeding	1548	10	2.3	78.1	97.3	19.1	
Second 4 days carotene feeding	1548	7	2.3	90.6	109.7	18.8	
Third 4 days carotene feeding	1548	5	2.3	85.8	103.4	17.6	
Last 4 days of depletion period	1709	9	3.5	107.6	124.3	16.7	260
First 4 days carotene feeding	1709	5	3.5	82.6	88.4	5.8	
Second 4 days carotene feeding	1709	4	3.5	89.7	90.2	0.5	
Third 4 days carotene feeding	1709	4	3.5	89.0	103.7	14.7	
Last 4 days of depletion period	1711	6	3.8	109.0	124.6	15.6	240
First 4 days carotene feeding	1711	6	3.8	92.8	111.0	18.1	
Second 4 days carotene feeding	1711	4	3.8	81.0	96.5	15.5	
Third 4 days carotene feeding	1711	7	3.8	88.5	105.7	17.2	

Table IV presents a summary of the biological assay data collected. It is evident from these data that 150,000 A.D.M.A. units of vitamin A in the form of carotene per cow per day for a twelve-day period did not bring about the expected increase in vitamin A potency. It is probable that a twelve-day period is insufficient time for the cows to secrete milk of normal vitamin A potency under such feeding conditions. Three of the four cows used in this study seemed to give the same response to intensive carotene feeding since there was a very slight increase in the vitamin A potency of their milks. The milk from Holstein cow No. 1709 during periods II and III actually showed a decrease in vitamin A content.

A wide difference in vitamin A potency is noted between the four milks studied. The milk from cow No. 1546 contained the greatest amount of vitamin A, approximately 453 units per quart, while the milks from cows Nos. 1548, 1709, and 1711 contained approximately 401, 261, and 242 vitamin A units per quart, respectively.

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* The commercial carotene was supplied gratis by the S.M.A. Corporation, Cleveland, Ohio.

The data obtained in the above studies agree with the findings of Fraps and coworkers (10), who reported that the feeding of 116,000 rat units (?) of vitamin A per day is not sufficient to maintain the vitamin A potency of butterfat. Baumann and Steenbock (8) reported that the feeding of 50 to 60 pounds of freshly cut green alfalfa daily caused the carotene and the vitamin A content of the butterfat to increase rapidly. This alfalfa contained approximately 1,000,000 (?) units of vitamin A and caused the milk to contain maximum values for both carotene and vitamin A within a two weeks' period. A paper by Loy, Hilton, Wilbur and Hauge (11) appeared just subsequent to the preparation of this manuscript. These authors draw the conclusion that it takes from 10 to 11 days for the milk fat of the cow to reach its vitamin A equilibrium when the animal is shifted from a vitamin A rich diet to a vitamin A poor diet, and vice versa. With these conclusions in mind, the data of the present study would seem to indicate that 150,000 A.D.M.A. units per day were only sufficient to maintain constant the vitamin A potency of milks of cows receiving a pigment poor diet. If these cows had been fed this amount of carotene for a longer period of time, milk with a higher vitamin A potency might have been produced.

IV. The vitamin A potency of milks produced by five standard dairy breeds under identical feeding conditions

In the previous studies, wide variations in vitamin A potency of milks were noted among individual cows of the same breed. To partially compensate for such individual variations within the breed, ten cows from each breed were chosen as experimental subjects in this phase of the investigation. These cows were selected and handled in a manner similar to those previously described.

Table V portrays a record of the average food consumption and milk production for the five breeds of cows used in these studies.

TABLE V

The average food consumption and milk production for the five breeds of cows used in this study

BREED	GRAIN CONSUMED DAILY	SILAGE CONSUMED DAILY	HAY CONSUMED DAILY	MILK PRODUCED DAILY	FAT PRODUCED DAILY	PER CENT FAT
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	
Holstein	11.9	29.5	8.3	39.2	1.403	3.57
Jerseys	7.1	20.8	8.3	18.6	1.146	6.15
Guernseys	7.9	22.3	8.3	22.0	1.178	5.35
Ayrshires	10.9	27.5	8.3	32.4	1.264	3.90
Brown Swiss	10.3	28.6	8.3	30.0	1.154	3.84

Pooled samples of milk from each of the breeds were taken daily for the purpose of biological assay. These pooled samples consisted of equal volumes of milk from each cow of the group during each of the milking periods. Measured aliquots of the pooled samples of milk from each of the breeds were fed to the experimental animals on the same day they were taken.

The results of the experiment relating to the vitamin A potency of milks produced by representative cows from five major dairy breeds (Table VI) indicate that there is an appreciable difference in vitamin A activity of the milk produced by Holstein, Brown Swiss, Ayrshire, Guernsey, and Jersey cows when maintained under identical nutritive conditions. These results are not in agreement with those of Davis and Hathaway (3) who concluded that there was no difference in vitamin A content of milk produced by different breeds under identical nutritive conditions.

Data obtained in this experiment indicate that there was no appreciable difference in the vitamin A activity of milk fat produced by the different breeds. These results are in agreement with those reported by Wilbur, Hilton, and Hauge (5) who concluded that there was no difference in vitamin A content of milk fat produced by Guernsey and Ayrshire cows.

CONCLUSIONS

(1) A study of the vitamin A potency of milk from Guernsey and Holstein cows receiving a normal dairy ration showed that individual variations occur within the breed, as well as between the breeds. In general, milks produced by Guernsey cows were higher in vitamin A than milks produced by Holstein cows.

(2) The vitamin A content of milks from Guernsey and Holstein cows which received a pigment-poor ration decreased approximately 70 per cent in seven months. The two breeds of cows responded quite uniformly in this regard under these adverse feeding conditions.

(3) When the cows, depleted of their vitamin A reserves, were subjected to high carotene feeding for a period of twelve days, the administration of 150,000 A.D.M.A. units per day was found to be insufficient to cause an increase in the vitamin A potency of the milks produced by the cows of either breed.

(4) Milks from groups of cows representing five major dairy breeds which were fed the same ration varied in vitamin A activity per unit volume. The vitamin A potency per gram of milk fat, however, was approximately the same for all breeds.

(5) It is concluded that the vitamin A potency of milks from the various dairy breeds is proportional to the percentage of milk fat characteristic of the breed.

TABLE VI

The vitamin A potency of milks produced by representative cows from five major dairy breeds

BREED	NUMBER OF RATS	DOSE CC.	WEIGHT OF RATS AT END OF FORE PERIOD GMS.	WEIGHT OF RATS AT END OF EXPERIMENT GMS.	GAIN IN WEIGHT GMS.	DAILY GAIN PER GRAM OF MILK FED	VITAMIN A UNITS PER QUART	VITAMIN A UNITS PER GRAM OF FAT (SHERMAN)
Holstein	7	0.6	97.1	109.0	11.9	0.70	1699	20
Holstein	16	1.0	108.0	135.4	27.4			
Brown Swiss	10	0.6	112.0	136.2	24.2	1.00	1980	25
Brown Swiss	17	1.0	108.0	139.9	31.9			
Ayrshire	11	0.6	113.2	137.2	24.0	1.04	2157	26
Ayrshire	15	1.0	114.4	149.1	34.7			
Guernsey	10	0.6	102.6	128.4	25.8	1.23	2708	22
Guernsey	13	1.0	107.6	151.2	43.6			
Jersey	14	0.6	112.4	139.0	26.6	1.39	3212	22
Jersey	14	1.0	99.5	151.2	51.7			

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The Avitaminoses. The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases. By Walter H. Eddy, Ph.D., Professor of Physiological Chemistry, Teachers College, Columbia University, and Gilbert Dalldorf, M.D., Pathologist to the Grasslands and Northern Westchester Hospitals, Westchester County, New York. The Williams and Wilkins Company, Mt. Royal and Guilford Aves., Baltimore, Maryland, 1937. 338 pp. Illustrated. 15.5 × 23.5 cm. Price, $4.50.

This volume, which contains a foreword by Dr. James Ewing, is an outgrowth of the "Vitamine Manual" published by the senior author in 1921. In the preface the authors state that it has been their desire to prepare "a helpful manual rather than a complete treatise," covering the more important developments concerning the nature and functions of the vitamins. The collaboration of Eddy and Dalldorf brings to the reader the viewpoints of a biochemist with a rich experience in the vitamin research field and the viewpoints of a physician experienced in laboratory and clinical research.

As the title indicates, the book stresses the pathology of vitamin deficiency, since it is by means of pathological studies and the production and cure of deficiency diseases that it is possible to obtain a clear idea of vitamin function.

Chapter I, consisting of eleven pages, is devoted to an introduction to the subject; while chapters II to VI, inclusive, consist of 46 pages devoted to vitamin A (and its parent substance carotene). In this small space the authors have condensed an unusually large amount of information regarding the chemistry and functions of vitamin A and vitamin A unitage as well as clinical, anatomical and subclinical manifestations of vitamin A deficiency.

Chapters VII to XV, inclusive, contain 92 pages devoted to the nature and functions of the recognized constituents of the vitamin B complex. The chemistry of B (B_1) and flavin are discussed briefly, while considerable space is devoted to the pathological and clinical aspects of beriberi and pellagra. A brief outline of the somewhat controversial evidence for the other B factors is also included, as well as a description of attempts at establishing units of measurement of vitamin potency.

Chapters XVI to XIX, inclusive, consist of 44 pages devoted to vitamin C with discussions pertaining to chemical properties and the configuration of ascorbic acid and anatomical, clinical and sub-clinical manifestations of scurvy.

Three chapters (XX, XXI and XXII), consisting of 34 pages, are devoted to vitamin D and rickets, while two chapters (XXIII and XXIV) consist of 14 pages devoted to vitamin E and vitamin E deficiency. The remaining chapters, XXV, XXVI and XXVII, contain brief discussions of such topics as the relation of "vitamins to resistance and infection," "morbid effects of certain complicated dietary experiments" and "vitamins and blood regeneration."

An appendix, designated as Part II, contains 30 pages covering such topics as "standard methods for vitamin bio-assay" and "clinical tests for evidence of vitamin deficiency in humans." A table of vitamin values for food is also included. The book contains author and subject indices.

The reviewer feels that the book would have been strengthened by more detailed discussions of certain topics. In an attempt at brevity, the authors have been forced to omit many important details of value. The book is illustrated with an unusually fine collection of photographs portraying pathological and clinical aspects of vitamin deficiency diseases. Each chapter, except those in Part II, is followed by bibliographical references to original literature, to which the reader may refer if further details are desired.

There are a number of typographical errors which will undoubtedly be corrected in future reprintings. These are confined largely to chemical formulas such as the formula for vitamin B_1 on page 69, for dehydroascorbic acid on page 167 and for ergosterol on page 212.

While "The Avitaminoses. The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases" cannot be considered a comprehensive treatise, it can be read with pleasure and profit by specialists working in the field of nutrition. The reviewer predicts that the book will find wide use by busy physicians, dietitians and students of nutrition because it contains a wealth of physiological and clinical material which can only be found in the original research literature.

R. Adams Dutcher

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THE LITTLE THINGS IN LIFE.
of Arkansas. D. Appleton
York City, 1937. xi + 340

The purpose of this book is
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The book is written for the
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[Reprinted from Journal of Chemical Education, Vol. 14, No. 8. August, 1937.]

THE LITTLE THINGS IN LIFE. *Barnett Sure, Ph.D.*, University of Arkansas. D. Appleton-Century Company, Inc., New York City, 1937. xi + 340 pp. 13.5 × 20.5 cm. $2.50.

The purpose of this book is to present, in non-technical language, "the story of the vitamins, mineral elements and ductless glands, in relation to nutrition and health of man." The author states that he has endeavored to stress the applications of scientific research to human problems and has purposely endeavored to minimize the laboratory phases so far as experimental animals are concerned.

The book is written for the lay reader in a clear and pleasing style that should appeal to non-technical readers. On the other hand, the reviewer feels that the book can be read with pleasure and profit by physicians and students of nutrition for the reason the author deals with many subjects which do not ordinarily come to the attention of the busy specialist.

Chapter I deals with deficiency diseases such as scurvy, Barlow's disease, rickets, and beriberi, with special reference to cause and dietary treatment.

Chapter II, entitled "The Vitamins," gives a brief historical résumé of the discovery of vitamins accompanied by a discussion of their occurrence, functions, properties, and stability. Theoretical and controversial topics are avoided quite successfully, and practical questions of interest to the layman are stressed.

Chapter III is devoted to a brief discussion of "Vitamins and Dental Disorders" while Chapter IV gives a brief discussion of "The Relation of Vitamin Deficiencies to Colds and Infections."

Chapter V treats of "Vitamins in Infant Nutrition" which is followed (Chapter VI) by a discussion of "Vitamins during the Nursing Period and Pregnancy."

Subsequent chapters (VII to XIV inclusive) deal with such topics as "Vitamins and Malnutrition," "Vitamins in Various Ailments," "Vitamin Content of Foodstuffs," "Minerals, Anemias and Goiter," "Foods, Hay-Fever and Asthma," "Ferments and Indigestion," "The Ductless Glands and Nutrition," and "The Little Things of Life Are the Big Things of Life."

Five pages at the end of the book are devoted to a glossary which gives simple definitions for technical terms used in the preceding chapters. Author and subject indices are also appended. The reviewer infers that this book is to be included in the Appleton Popular Health Series to which many eminent popular writers on health and medical topics have contributed.

Dr. Sure writes with authority in the vitamin field, in which he has made many important contributions. He writes convincingly in related fields, in which he has done little or no experimental work. In the reviewer's opinion Dr. Sure has done a remarkably good job in reviewing the clinical field, where he has had little or no first-hand experience. It is entirely probable that some members of the medical fraternity will feel that Dr. Sure has overstepped, at times, that debatable zone between biochemistry and medicine which has been guarded somewhat jealously by the medical profession. In most cases, however, the author defends his position by direct references to the original experimental and clinical literature.

The reviewer knows of no popular treatise which discusses the topics enumerated with greater clarity and interest.

R. ADAMS DUTCHER

THE PENNSYLVANIA STATE COLLEGE
STATE COLLEGE, PENNSYLVANIA

Reprinted from SCIENCE, O

THE FERMENTATION OF CIGAR-L
TOBACCO[1]

CONTRARY to the observations of Loew,[2] i
tions at the Pennsylvania Agricultural Ex
Station emphasize the significance of microor
in the normal fermentation of cigar-leaf tobac
epiphytic flora, consisting chiefly of member
genera *Bacillus, Staphylococcus, Aspergillus,*
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Bacillus were found to multiply rapidly; ag
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culiar variation of *B. subtilis,* or it may be
as a previously undescribed species. The
organism is a slender, motile rod, forming c
excentric spores. The response of this ba
biochemical tests corresponds closely to the
of *B. subtilis.* Colonies upon agar are sprea
herent and somewhat mycoides-like.

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[1] Authorized for publication on July 29, 1937,
No. 781 in the Journal Series of the Pennsylva
cultural Experiment Station.

[2] O. Loew, "Curing and Fermentation of C
Tobacco," U. S. Dept. Agr. Rep't. 59, 1899;
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Tobacco Plant," U. S. Dept. Agr. Rep't. 68, 19

Reprinted from Science, October 29, 1937, Vol. 86, No. 2235, page 404.

THE FERMENTATION OF CIGAR-LEAF TOBACCO[1]

Contrary to the observations of Loew,[2] investigations at the Pennsylvania Agricultural Experiment Station emphasize the significance of microorganisms in the normal fermentation of cigar-leaf tobacco. An epiphytic flora, consisting chiefly of members of the genera *Bacillus, Staphylococcus, Aspergillus, Penicillium, Rhizopus* and *Mucor,* was found upon the cured leaf. During the fermentation, species of the genus *Bacillus* were found to multiply rapidly; agar plate counts of these organisms increased from values of less than 500,000 per gram to values in excess of 2,000,000,000 per gram. Direct counts revealed increases from an initial figure of less than 500,000,000 per gram to counts greater than 15,000,000,000 per gram. The rate of multiplication depended, among other factors, upon the quality of the substrate, the amount of moisture present and the temperature maintained. Although members of this genus were present in the spore state at the end of the curing process, chains of vegetative cells of this group appeared in the early stages of the subsequent fermentation. Two types take part in this extensive multiplication of spore-formers. One type was easily identified as *B. megatherium.* The latter organism represents either a peculiar variation of *B. subtilis,* or it may be classified as a previously undescribed species. The second organism is a slender, motile rod, forming central to excentric spores. The response of this bacillus to biochemical tests corresponds closely to the reactions of *B. subtilis.* Colonies upon agar are spreading, adherent and somewhat mycoides-like.

Staphylococci were frequently observed upon cured and fermenting tobacco. Occasionally the numbers equaled or exceeded those of bacilli, but in many cases the normal fermentation progressed without the appearance of this organism; on the other hand, bacilli were always found in the fermenting tobacco.

Although present in significant numbers on cured tobacco, members of the genera *Aspergillus, Penicillium, Mucor* and related types were found to decrease in number during the course of the fermentation. It was found, however, that an acid-agar medium was of value in the study of cured and curing tobacco. The predominance of *Aspergilli* and *Penicillia* on these plates seemed to indicate a tobacco difficult to ferment. This, apparently, is associated with the degree of complexity of carbohydrate and nitrogenous material in the leaf.

Studies of the catalase activity of the tobacco revealed a direct relation between the number and the activities of the microorganisms on the leaf. Low bacterial counts were invariably accompanied by slight catalase activity and high counts by considerable catalase activity. Any experimental treatment of the leaf that resulted in the inhibition of bacterial growth prevented increases in catalase activity. Any increase in catalase activity was accompanied by an increase in bacterial numbers. Catalase activity was restored to tobacco rendered inactive by heat treatment when inoculated with cultures previously isolated from tobacco.

This work will be reported in detail in a series of technical bulletins of the Pennsylvania Agricultural Experiment Station.

J. J. Reid
D. W. McKinstry
D. E. Haley

Pennsylvania Agricultural
Experiment Station,
State College, Pa.

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[1] Authorized for publication on July 29, 1937, as paper No. 781 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] O. Loew, "Curing and Fermentation of Cigar-Leaf Tobacco," U. S. Dept. Agr. Rep't. 59, 1899; "Physiological Studies of Connecticut Leaf Tobacco," U. S. Dept. Agr. Rep't. 65, 1900; "Catalase, A New Enzyme of General Occurrence, with Special Reference to the Tobacco Plant," U. S. Dept. Agr. Rep't. 68, 1901.

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 34, No. 4, October 1937

PROCEEDINGS OF LOCAL BRANCHES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

CENTRAL PENNSYLVANIA BRANCH

BUCKNELL UNIVERSITY, LEWISBURG, PENNSYLVANIA, MAY 15, 1937

Syphilis from the Standpoint of the Clinician. *Peter P. Mayock*, Chief of State Genito-Urinary Clinic, Wilkes-Barre, Pennsylvania.

Social Work and Syphilis. *Nellie Loftus*, Chief Nurse and Social Worker, State Clinic, Wilkes-Barre, Pa.

Stenosing Proctitis, or the Sixth Venereal Disease. *G. N. Fleugel*, State Genito-Urinary Clinic, Wilkes-Barre, Pennsylvania.

Modern Problems in the Experimental Study of Syphilis. *Harry Eagle*, United States Public Health Service, Washington, D. C. and the Johns Hopkins Medical School, Baltimore, Maryland.

Contrary to the accepted teaching of 25 years, it is questionable whether the *Spirocheta pallida* has ever been successfully cultivated on artificial media. Such regular cultivation of a truly pathogenic *Spirocheta pallida* is perhaps the most pressing problem in syphilis research.

In the serodiagnosis of syphilis, it is important that serologists devise new, more sensitive and more reliable modifications of the laboratory tests; but it is even more important that laboratory technicians learn to use properly those technics which are already available.

The nature of the reactive substance in syphilitic serum has been a long-standing mystery. It seems a plausible working hypothesis, consistent with the known facts, that it is simply an antibody to the *Spirocheta pallida*. That this antibody reacts with a non-specific antigen (e.g., beef heart lipoid) may simply be due to the presence in the spirochete and in mammalian tissue of a serologically related antigenic factor, a cross-reaction for which there are numerous analogies.

The nature of the "immunity" (i.e., resistance to re-infection) engendered by syphilitic infection is as yet wholly obscure. The elucidation both of the biologic properties of the *Spirocheta pallida*, and of the nature of the antibody response in the infected individual, have been seriously handicapped by our inability to grow the organism *in vitro*.

With respect to the chemotherapy of syphilis, although the arsphenamines constitute a therapeutic agent of proved value, the mechanism of their action is as yet obscure. The elucidation of this problem may well provide a clue to the development of more effective, more rapid or safer methods of treatment.

Tuberculous Tracheobronchitis. Preliminary Report of its Treatment by Electrocoagulation. *F.*

W. Davison, Department of Otolaryngology and Bronchoscopy, The George F. Geisinger Memorial Hospital, Danville, Penn.

The pathology of tuberculous tracheobronchitis has been well described by Dr. Leo Eloesser. Accurate diagnosis of these lesions is not possible without bronchoscopic examination and it is only recently that the bronchoscope has been used as a means of diagnosis in tuberculous patients. During the past nine months we have had the opportunity of observing and treating three patients with tuberculous, tracheal and bronchial lesions. While the number of patients treated is small, the results have been so consistently satisfactory that I think it is worth while to make a preliminary report with the hope that others will give this method a trial.

I have been using an electro-coagulation current of low intensity, not with the idea of destroying all the tuberculous tissue present, but with the idea of inducing an acute inflammatory reaction locally, which results in increased blood supply and in hastening production of fibrous tissue.

No harmful effects were noted as a result of bronchoscopic examination and in each case the tracheo-bronchial lesions showed a consistent tendency to heal under the influence of treatment with a mild electro-coagulation current.

THE RELATION OF CATALASE ACTIVITY TO THE MICROFLORA OF CURED AND FERMENTING TOBACCO. *J. J. Reid, D. E. Haley, D. W. McKinstry and J. D. Surmatis*, Division of Bacteriology and Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pa.

An extensive flora was found on the leaves of cured Pennsylvania cigar-leaf tobacco. Spores of members of the genus *Bacillus*, cocci, and fungi of the genera *Aspergillus*, *Pencillium* and *Rhizopus* comprised the major portion of the microflora present. Tobacco of poor quality showed fewer bacteria than tobacco of good quality, although in some cases the numbers of fungi present were greater than in the case of the better grades of tobacco.

Catalase activity of the cured tobacco was found to be directly related to the numbers of microorganisms present. High bacterial counts were invariably associated with marked catalase activity and low counts with little or no activity.

During the fermentation process a marked increase in bacterial numbers was noted and this in every instance was accompanied by a relative increase in catalase activity. Vegetative cells of the genus *Bacillus*, occurring in chains of from 2 to 8 organisms, were observed.

Any treatment of tobacco attempted which was found to inhibit the reproduction of microorganisms, likewise prevented an increase in catalase activity. Treatments, on the other hand, that resulted in an increase in bacterial numbers, resulted in an increase in catalase activity.

Normal catalase activity was restored to tobacco rendered inactive by heat by means of inoculation with pure cultures of bacilli isolated from cured and fermenting tobacco.

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(1) Authorized for publicati
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(2) Longenecker and Haley,
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N. J., for a generous supply of
(4) Nicloux, *Mem. Soc. Biol.*
(5) Armstrong and Ormero
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(6) Noyes, Lorberblatt and
(7) Expressed in ml. 0.1 *N*

[Reprint from the Journal of the American Chemical Society, **59**, 2156 (1937).]

[CONTRIBUTION FROM THE LABORATORY OF THE DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY, THE PENNSYLVANIA STATE COLLEGE]

Further Studies on the Nature of Ricinus Lipase and its Action[1]

BY HERBERT E. LONGENECKER AND D. E. HALEY

Considerable recent progress has been made along special lines of enzyme research, especially in the elucidation of the nature and mode of action of enzymes. This progress has been witnessed in connection with nearly all except the fat splitting enzymes. Ricinus lipase, the lipolytic principle of the castor bean (*Ricinus communis*) is the most highly active of this particular group. It, however, has received the least attention during the recent advances in our knowledge of enzymes. This fact is probably due to a variety of underlying reasons chief of which is the discouraging sensitivity of most workers to the dried fat free castor bean preparations.

The present communication represents an attempt to contribute to the existing knowledge of the nature of the lipolytically active principle of the castor bean and the mode of its action.

Experimental

A. Materials and Methods.—The experimental procedures and techniques followed in these studies were essentially the same as those previously reported.[2] The considerable husk on the castor beans[3] was removed prior to extraction in all cases except where a specific experiment was conducted to redetermine the necessity of this operation[4–6] (*cf.* Table II).

Method of Calculation of Lipolytic Activity.—A formula for the calculation of the percentage hydrolysis of an oil is given by Longenecker and Haley.[2] Through a typographical error in the original paper, the term (× 100) in the formula was omitted. The correct expression of the formula is

$$\frac{\text{ml. } 0.1\ N \text{ alkali (sample)} - \text{ml. } 0.1\ N \text{ alk. (blank)}}{\text{Saponification no.}^{7} - \text{Free fatty acid no.}^{7}} \times 100 = \%\ \text{hydrolysis}$$

A standard procedure was adopted for the estimation of lipolytic activity. This was followed unless mention is made of a special procedure in some of the following experiments. This "standard determination" consisted of introducing definite weights of olive oil and lipase material into 5-ml. hard glass bottles which were then brought to the proper temperature (37–38°). Acetic acid (0.1 *N*) was next added to provide the necessary water for the reaction and to bring the *p*H of the mixture to the optimum.[8] The same sample of olive oil was used throughout the work reported in this paper.

While the formula mentioned above permits the calculation of the percentage hydrolysis, it is desirable at times to know the relative activity of different preparations as compared to each other. To express this activity an arbitrary measure, the lipase unit (L. U.) has been derived.[9] The formula $1000/WT$ = L. U., in which T is the time of reaction in minutes required to effect a 40% hydrolysis of olive oil (saponification no. 190), and W is the weight of the enzyme preparation in grams.

The time required for a 40% hydrolysis may best be determined by plotting the course of the reaction against time and determining the point at which 40% of the material is hydrolyzed. The point of 40% hydrolysis was chosen as the basis of calculations after a number of observations indicated that the cumulative effects of contact with the digestion mixture (discussed later) were not appreciable until some time after 50–60% of the olive oil had hydrolyzed. Typical results from the calculations of the lipase units in two active preparations are shown in Table I.

TABLE I

ENZYME UNITAGE OF TWO TYPICAL RICINUS LIPASE PREPARATIONS

Wt. lipase material, g.	Time for 40% hydrolysis, min.	Lipase units, L. U.
Preparation 13a, passed 20-mesh sieve		
0.100	16	625
.080	19.5	636
.070	23	621
.060	26	641
.050	38	526
.040	51	500
Preparation 13b, passed 40-mesh sieve		
0.100	11	909
.080	15	890
.060	19.5	900
.050	22	910
.040	30	825

Effect of Decortication on Lipase Activity.—A large number of whole beans was subdivided into two samples; from one the outer husk was removed prior to the extraction, while the other was treated in the usual manner except that the husk was not removed. The relative activities of the two samples are shown in Table II.

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[1] Authorized for publication on January 16, 1937, as paper No. 757 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the Pennsylvania State College.

[2] Longenecker and Haley, THIS JOURNAL, **57**, 2019 (1935).

[3] We are indebted to The Baker Castor Oil Co. of Newark, N. J., for a generous supply of large size Manchurian beans.

[4] Nicloux, *Mem. Soc. Biol.*, **56**, 701, 839, 868 (1904).

[5] Armstrong and Ormerod, *Proc. Roy. Soc.* (London), **78B**, 376 (1906).

[6] Noyes, Lorberblatt and Falk, *J. Biol. Chem.*, **68**, 135 (1926).

[7] Expressed in ml. 0.1 *N* alkali.

[8] Haley and Lyman, THIS JOURNAL, **43**, 2664 (1921).

[9] The suggestions of Dr. E. S. Flinn have been helpful in this portion of the work.

TABLE II

COMPARISON OF ACTIVITIES OF RICINUS LIPA
WHOLE AND DECORTICATED BEANS

Time of digestion, min.	Hydrolysis, % A	B
6	..	28.
10	39.7	40.
15	33.5	53.
30	76.3	72.
45	82.1	81.
60	85.8	86.
120	89.0	92.
180	90.7	.
300	92.7	.

Effect of Fat Solvent on Lipase Activity.—Sin
observed that the extraction of the beans procee
rapidly with some solvents than with others, the
of bean preparations extracted with ethyl ether,
and a carefully fractionated (b. p. 32–34°) petrole
were compared with those extracted with ordi
troleum ether (b. p. 20–40°). In all cases 1.0 g.
oil, 0.100 g. of lipase and 0.6 ml. of 0.1 *N* acetaldeh
used. The results are shown in Table III.

TABLE III

PERCENTAGE HYDROLYSIS OF OLIVE OIL WITH
LIPASE PREPARED WITH VARIOUS SOLVEN

Solvent used	Time of digestio 1	4
Petroleum ether (20–40°)	59.0	81.3
Petroleum ether (32–34°)	64.2	82.4
Ethyl ether	22.1	52.3
Acetone	20.7	41.1

Effect of Different Amounts of Water and Oil
ent Lipase Activity.—To determine the optimum
for lipase activity, the amounts and proportion
oil and water in the digestion mixture were varied,
in Table IV. The quantities of lipase material
of 417 L. U.), and acetic acid (equivalent to 0.6

TABLE IV

ACTION OF RICINUS LIPASE IN THE PRESENCE O
CONCENTRATIONS OF OLIVE OIL AND WA

Composition digestion mixture Oil, g.	Water, g.	E. m. f.25°, −mv.	Hours 1 Hydrolysis, %	5
1.00	0.02	...	4.4	14.0
1.00	.03	...	16.9	30.0
1.00	.05	170	30.6	49.3
1.00	.06	172	35.5	49.8
1.00	.10	172	43.0	65.9
1.00	.20	173	50.6	74.0
1.00	.30	170	53.6	81.8
1.00	.60	172	55.8	73.9
1.00	1.00	172	50.1	75.6
1.00	2.00	180	42.8	74.7
1.00	3.00	190	40.2	68.0
0.50	0.60	175	78.1	100.0
1.00	0.60	172	56.1	73.9
1.50	0.60	172	40.0	68.0
2.00	0.60	172	34.8	60.7
3.00	0.60	178	18.7	52.0

The effect of oleic acid
ration is thus apparent.
five series of samples were
in Fig. 2. In all series,
(670 L. U.) and 0.6 ml.
In series A, 1.00 g. of oli
a standard determination;
was used. In series C the
allowed to proceed for tw
additional 1.00 g. of oil wa
for three minutes, and t
mined in the usual fash
similar in all respects to s
twenty-four hours a thir
added, and the mixture a
twenty-four hour period,
drolysis was determined
sults are expressed on the

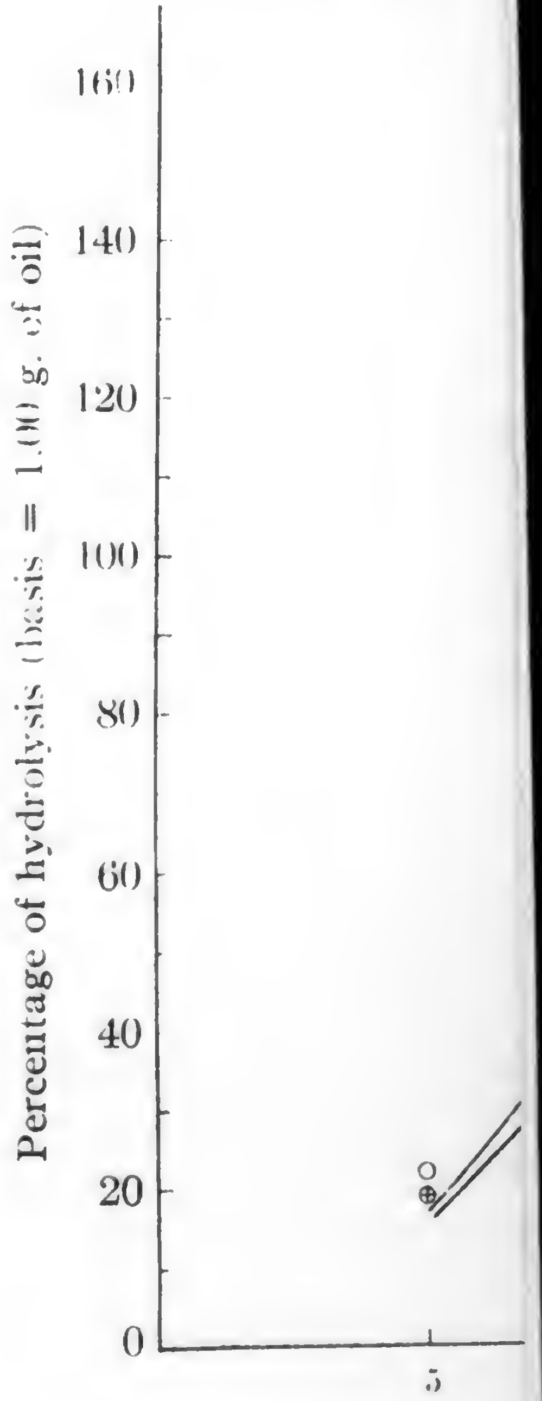

Fig. 2.—The effect on li

Dis

The arbitrary meth
activity suggested in t
similarly employed in t
It considerably simpli
large numbers of data
of rates of action of v
effect of a particular
quantities of lipase pre
the time required for 4

[10] Northrup, *J. Gen. Physi*

[Reprint from the Journal of the American Chemical Society, **59**, 2156 (1937).]

[CONTRIBUTION FROM THE LABORATORY OF THE DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY, THE PENNSYLVANIA STATE COLLEGE]

Further Studies on the Nature of Ricinus Lipase and its Action[1]

BY HERBERT E. LONGENECKER AND D. E. HALEY

Considerable recent progress has been made along special lines of enzyme research, especially in the elucidation of the nature and mode of action of enzymes. This progress has been witnessed in connection with nearly all except the fat splitting enzymes. Ricinus lipase, the lipolytic principle of the castor bean (*Ricinus communis*) is the most highly active of this particular group. It, however, has received the least attention during the recent advances in our knowledge of enzymes. This fact is probably due to a variety of underlying reasons chief of which is the discouraging sensitivity of most workers to the dried fat free castor bean preparations.

The present communication represents an attempt to contribute to the existing knowledge of the nature of the lipolytically active principle of the castor bean and the mode of its action.

Experimental

A. Materials and Methods.—The experimental procedures and techniques followed in these studies were essentially the same as those previously reported.[2] The considerable husk on the castor beans[3] was removed prior to extraction in all cases except where a specific experiment was conducted to redetermine the necessity of this operation[1–6] (*cf.* Table II).

Method of Calculation of Lipolytic Activity.—A formula for the calculation of the percentage hydrolysis of an oil is given by Longenecker and Haley.[2] Through a typographical error in the original paper, the term (× 100) in the formula was omitted. The correct expression of the formula is

$$\frac{\text{ml. } 0.1\ N \text{ alkali (sample)} - \text{ml. } 0.1\ N \text{ alk. (blank)}}{\text{Saponification no.}^{7} - \text{Free fatty acid no.}^{7}} \times 100 = \%\ \text{hydrolysis}$$

A standard procedure was adopted for the estimation of lipolytic activity. This was followed unless mention is made of a special procedure in some of the following experiments. This "standard determination" consisted of introducing definite weights of olive oil and lipase material into 5-ml. hard glass bottles which were then brought to the proper temperature (37–38°). Acetic acid (0.1 *N*) was next added to provide the necessary water for the reaction and to bring the *p*H of the mixture to the optimum.[8] The same sample of olive oil was used throughout the work reported in this paper.

While the formula mentioned above permits the calculation of the percentage hydrolysis, it is desirable at times to know the relative activity of different preparations as compared to each other. To express this activity an arbitrary measure, the lipase unit (L. U.) has been derived.[9] The formula $1000/WT$ = L. U., in which T is the time of reaction in minutes required to effect a 40% hydrolysis of olive oil (saponification no. 190), and W is the weight of the enzyme preparation in grams.

The time required for a 40% hydrolysis may best be determined by plotting the course of the reaction against time and determining the point at which 40% of the material is hydrolyzed. The point of 40% hydrolysis was chosen as the basis of calculations after a number of observations indicated that the cumulative effects of contact with the digestion mixture (discussed later) were not appreciable until some time after 50–60% of the olive oil had hydrolyzed. Typical results from the calculations of the lipase units in two active preparations are shown in Table I.

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0.100	11	909
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Effect of Decortication on Lipase Activity.—A large number of whole beans was subdivided into two samples; from one the outer husk was removed prior to the extraction, while the other was treated in the usual manner except that the husk was not removed. The relative activities of the two samples are shown in Table II.

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Time of digestion, min.	Hydrolysis, % A	B
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10	39.7	40.0
15	33.5	53.0
30	76.3	72.9
45	82.1	81.7
60	85.8	86.0
120	89.0	92.6
180	90.7	. .
300	92.7	. .

Effect of Fat Solvent on Lipase Activity.—Since it was observed that the extraction of the beans proceeded more rapidly with some solvents than with others, the activity of bean preparations extracted with ethyl ether, acetone and a carefully fractionated (b. p. 32–34°) petroleum ether were compared with those extracted with ordinary petroleum ether (b. p. 20–40°). In all cases 1.0 g. of olive oil, 0.100 g. of lipase and 0.6 ml. of 0.1 *N* acetaldehyde were used. The results are shown in Table III.

TABLE III

PERCENTAGE HYDROLYSIS OF OLIVE OIL WITH RICINUS LIPASE PREPARED WITH VARIOUS SOLVENTS

Solvent used	Time of digestion, hrs. 1	4	24
Petroleum ether (20–40°)	59.0	81.3	90.2
Petroleum ether (32–34°)	64.2	82.4	95.6
Ethyl ether	22.1	52.3	72.0
Acetone	20.7	41.1	57.0

Effect of Different Amounts of Water and Oil on Apparent Lipase Activity.—To determine the optimum conditions for lipase activity, the amounts and proportions of olive oil and water in the digestion mixture were varied, as shown in Table IV. The quantities of lipase material (0.100 g. of 417 L. U.), and acetic acid (equivalent to 0.6 ml. of 0.1 *N*) were kept constant in this experiment, and the e. m. f. of the solutions determined with a quinhydrone electrode to assure the proper *p*H.

TABLE IV

ACTION OF RICINUS LIPASE IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF OLIVE OIL AND WATER

Composition digestion mixture Oil, g.	Water, g.	E. m. f.$_{25°}$, −mv.	Hours 1 Hydrolysis, %	5	24
1.00	0.02	. . .	4.4	14.0	. .
1.00	.03	. . .	16.9	30.0	37.6
1.00	.05	170	30.6	49.3	60.5
1.00	.06	172	35.5	49.8	59.7
1.00	.10	172	43.0	65.9	74.3
1.00	.20	173	50.6	74.0	86.6
1.00	.30	170	53.6	81.8	91.5
1.00	.60	172	55.8	73.9	94.2
1.00	1.00	172	50.1	75.6	93.4
1.00	2.00	180	42.8	74.7	92.9
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0.50	0.60	175	78.1	100.0	. .
1.00	0.60	172	56.1	73.9	94.2
1.50	0.60	172	40.0	68.0	. .
2.00	0.60	172	34.8	60.7	. .
3.00	0.60	178	18.7	52.0	. .

Effect of Water on Lipase Activity.—To demonstrate the inactivation of lipase preparations by water two series of samples were treated under the following conditions. In series A, 0.100 g. of lipase preparation (910 L. U.) was placed in 0.5 ml. of carbonate-free distilled water at 37°, and held at this temperature for different lengths of time. At the end of the holding period 1.00 g. of olive oil and 0.1 ml. of 0.6 *N* acetic acid was added and the percentage hydrolysis determined. In series B the same amount of lipase preparation was placed in 1.00 g. of olive oil and 0.5 ml. of carbon dioxide-free distilled water and held at 37° for varying lengths of time. At the end of the holding period 0.1 ml. of 0.6 *N* acetic acid was added likewise, and the percentage hydrolysis determined. No correction was necessary for the action of the enzyme with water alone. Fig. 1 shows the results of this experiment.

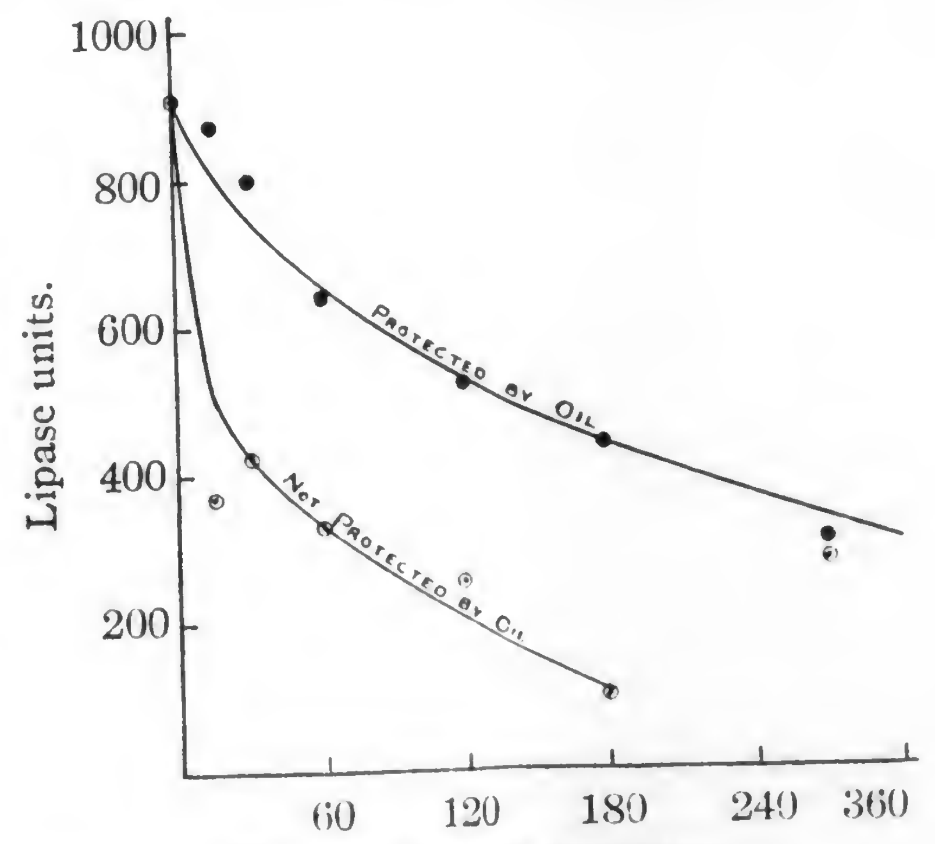

Fig. 1.—The effect of water on lipase activity.

Effect of Contact with Substrate and End-Products on Lipase Activity.—It has long been known that adsorption complexes may be formed between the enzyme and its substrate and between the enzyme and the end-products of the reaction. Such adsorption tends to inactivate the enzyme. In the case of Ricinus lipase and olive oil, the end-products are chiefly oleic acid and glycerol.

In preliminary experiments, the effect of oleic acid, olive oil and glycerol on the lipase were studied in the following manner: 2-g. samples of a lipase preparation (417 L. U.) were shaken at intervals during two days with 20 ml. each of the three materials. The undissolved material was filtered off and the residues from the oleic acid and olive oil were extracted separately with petroleum ether (b. p. 30–32°). The glycerol residues were not workable. The preparations thus obtained were used in standard determinations of five and one-half hours duration, together with a sample of untreated lipase preparation as a control. The results were as follows

Treatment	% Hydrolysis
Control	85
Oleic acid	52
Olive oil	75

The effect of oleic acid in inactivating the lipase preparation is thus apparent. A further test was made in which five series of samples were studied. The results are shown in Fig. 2. In all series, 0.100 g. of lipase preparation (670 L. U.) and 0.6 ml. of 0.1 *N* acetic acid were used. In series A, 1.00 g. of olive oil was used and constituted a standard determination; in series B, 2.00 g. of olive oil was used. In series C the reaction with 1.00 g. of oil was allowed to proceed for twenty-four hours, after which an additional 1.00 g. of oil was added, the bottles being shaken for three minutes, and the percentage hydrolysis determined in the usual fashion at intervals. Series D was similar in all respects to series C except that at the end of twenty-four hours a third portion of 1.00 g. of oil was added, and the mixture allowed to react for an additional twenty-four hour period, after which the percentage hydrolysis was determined as in the other series. The results are expressed on the basis of 1.00 g. of olive oil.

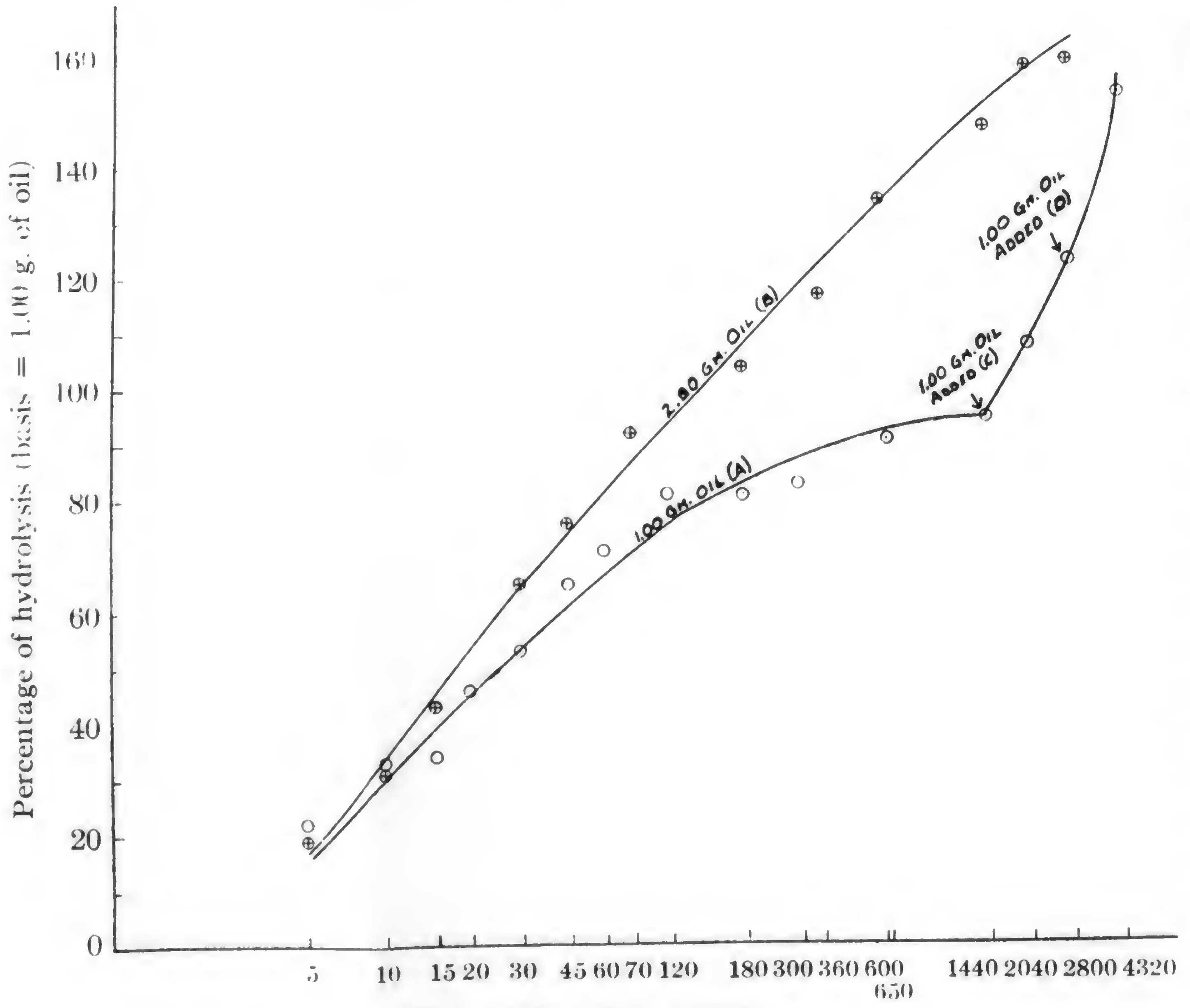

Fig. 2.—The effect on lipase activity of contact with substrate and end-product.

Discussion

The arbitrary method of calculating lipolytic activity suggested in this paper is an application similarly employed in the study of other enzymes.[10] It considerably simplifies the task of analyzing large numbers of data either for the comparison of rates of action of various preparations or the effect of a particular treatment. When small quantities of lipase preparation were used or when the time required for 40% hydrolysis was greater than twenty-five to thirty minutes the results appeared to be low, otherwise the method gives consistent results.

Preparations of the same high degree of activity have been obtained from whole and decorticated beans. This fact is of considerable importance in the large scale preparation of lipase-containing material, since the removal of the seed coats is a tedious process. It has been found that lipase preparations with an activity of 900–1000 lipase units could be prepared readily when extraction of the oil from the testing beans was carried out in glass equipment with low boiling petroleum ether. It is to be noted that the toxic nature of the castor bean[9–11] is intensified in the fat free preparations described here. It was necessary to observe constant caution to avoid physical contact with the dried extracted powder. All grinding and sieving operations were carried out in a closed box and a dust mask was worn whenever the preparations were handled.

Water was found to be necessary in quantities slightly exceeding the theoretical (0.06 g. per gram of olive oil) for maximum hydrolysis. The striking fact revealed by this set of experiments (Table IV) is that the fat hydrolysis is catalyzed to a considerable extent in the presence of the theoretical amount of water or less. There was hydrolysis in the presence of only a trace of water. This suggests the utility of the lipase to the germinating seed which usually has only small quantities of water for its divers reactions.

The percentage hydrolysis was higher in those cases where there was less oil per unit weight of enzyme. This supports the argument that a definite combination of enzyme and substrate does take place with Ricinus lipase. It is clear from these data that the rate of reaction is dependent upon the substrate concentration and not on the concentration of water in the digestion mixture. Such evidence is of importance in a consideration of the kinetics of the reaction, a report upon which will be made in a future communication.

The nature of the lipase material used in these experiments has been called to attention.[2] The preparations were solid mixtures consisting largely of protein and ash. It would be preferable to confine the term Ricinus lipase to the chemical individual which shows intensive and extensive lipolytic activity; as it is used here, it refers only to a sieved preparation of fat-free, castor beans. Attempts to isolate or even concentrate more active materials from these preparations yielded no positive results. The chief difficulty in these attempts seemed to lie in the marked effect of water on the activity of the enzyme, as is shown above (*cf.* Fig. 1).

While no evidence has been obtained to indicate the nature of this action of water on the enzyme, the presence of proteolytic as well as oxidative enzymes in our preparations may offer the clue. It is proposed to investigate these and other possibilities.

The effect of contact with the digestion mixture on the active enzyme probably represents the cumulative effects of water and the end-products formed during the reaction. The slight increase in hydrolysis on addition of substrate indicates that the enzyme has been inactivated but not entirely so after twenty-four hours contact with these substances. There was little additional hydrolysis of more substrate added after a second twenty-four hour period. These figures are interesting in contrast with the results obtained on the same amount of substrate in contact with the enzyme from the beginning of the reaction. Lack of a preparation of comparable unitage prevented following the course of this inactivation as would have been desirable after thirty, forty-five, sixty and one hundred and twenty minutes of contact with the digestion mixture.

Summary

In a continuation of studies on Ricinus lipase obtained from castor beans it has been found unnecessary to shell the beans prior to their extraction, extraneous material being removed while sieving at a later stage during the preparation. Low boiling petroleum ether proved the most satisfactory solvent for the preparation of very active samples. Reference has been made to dangers in handling these fat-free castor bean preparations.

An arbitrary expression for comparing the activities of preparations or the effect of various treatments has been presented. It is based on the time required for 40% hydrolysis. Limitations in the use of this lipase unitage basis are discussed.

Variation of the substrate concentration and the amount of water in the digestion mixture has shown the optimum conditions for Ricinus lipase action and the variance permissible for considerable action. Lipolytic action in the presence of a trace of water suggests the utility of the enzyme to the germinating seed. The dependence of the rate of reaction on the substrate concentration and not on the amount of water, except in quantities approximating the theoretical required, is discussed.

The inactivating effect of water and the end-products of the reaction have been shown. Inactivation of Ricinus lipase by water was found to take place in the presence and absence of oil.

STATE COLLEGE, PENNA.

RECEIVED JULY 16, 1937

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(10) Northrup, *J. Gen. Physiol.*, **16**, 41 (1932).

(11) Dowgard, *J. Am. Pharm. Assoc.*, **12**, 116 (1923).

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[Contribution from the Laboratory of th
Penn

The Effect of Certain Salts an

By Herbert F

Ricinus lipase obtained by any method
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than acids was indicated in the early st
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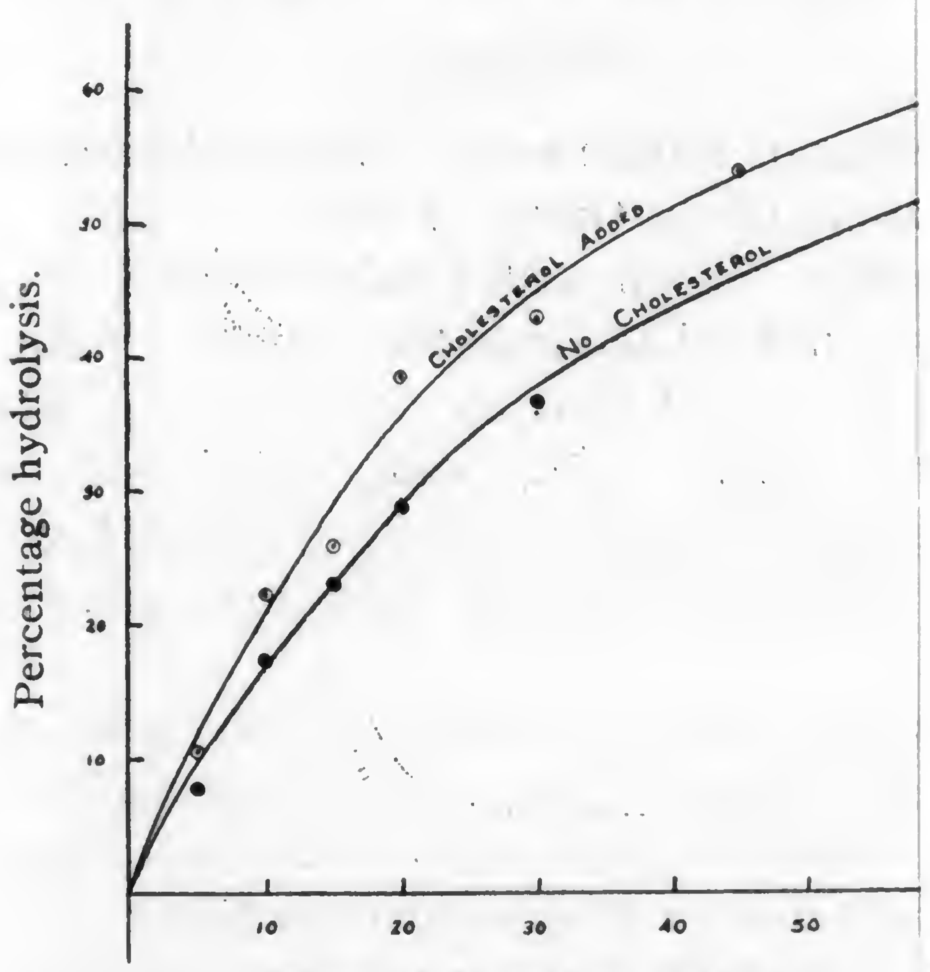

Fig. 1.—The effect of cholesterol on the act
Ricinus lipase.

were said to have a stimulating action
ganous sulfate has received especial atte
an activator for Ricinus lipase.[4–9]. Ot
stances which have been stated to af
catalysis of fat cleavage are amino ac
polypeptides and proteins,[12] alkaloids,[1

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(1) Authorized for publication on January 16, 193
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(2) Haley and Lyman, This Journal, **43**, 2664 (192

(3) Nicloux, *Mem. Soc. Biol.*, **56**, 701, 837, 868 (1904).

(4) Tanaka, *8th Intern. Congr. Appl. Chem.*, **11**, 37 (19

(5) Falk and Hamlin, This Journal, **35**, 210 (1913).

(6) Falk, *ibid.*, **35**, 601 (1913).

(7) Sudborough and Watson, *J. Indian Inst. Sci.*, **5**,

(8) Takamiya, *J. Agr. Chem. Soc.* (Japan), **5**, 595 (19

(9) Keeser, *Arch. exptl. Path. Pharm.*, **160**, 663 (1931).

(10) Hamlin, This Journal, **35**, 1897 (1913).

(11) Willstätter and Waldschmidt-Leitz, *Z. physiol.*
161 (1924).

(12) Falk, *J. Biol. Chem.*, **96**, 53 (1932).

(13) Padoa, *Nature*, **129**, 683 (1932).

(14) Padoa and Spada, *Giorn. biol. applicata ind. c*
(1931); *ibid.*, **3**, 121 (1933).

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Effect of Inorganic Sa
In each experiment, 1
preparation (L. U. = 910
containing 100 mg. of the
gether for three minutes
37–38°.

Salt added
None (check)
Mercuric acetate
Mercuric chloride
Cupric acetate
Cobalt acetate
Ferric chloride
Zinc chloride
Lead acetate
Lead chloride
Mercurous chloride
Chromium chloride
Nickel chloride
Calcium acetate
Manganese acetate
Manganese chloride
Ferric acetate
Chromium acetate

tion was not so marked in
note that the preparatio
contain 7.15–7.25% ash w
been shown to contain ma
iron.[23] These naturally o
marked effect on the lip
fairly high concentration.
findings of Willstätter an
ganous salts had no stimu
ditions of activity.

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(23) We are indebted to Mr

[Reprinted from the Journal of the American Chemical Society, **59**, 2160 (1937).]

[CONTRIBUTION FROM THE LABORATORY OF THE DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY, THE PENNSYLVANIA STATE COLLEGE]

The Effect of Certain Salts and Cholesterol on the Activity of Ricinus Lipase[1]

BY HERBERT E. LONGENECKER AND D. E. HALEY

Ricinus lipase obtained by any method thus far proposed does not catalyze the hydrolysis of fats or oils until it has first been activated. A variety of substances had been used to activate the enzyme before the realization of optimum conditions for its action.[2] The effect of substances other than acids was indicated in the early studies of Nicloux.[3] Sulfates of manganese and calcium were said to have a stimulating action. Manganous sulfate has received especial attention as an activator for Ricinus lipase.[4–9] Other substances which have been stated to affect the catalysis of fat cleavage are amino acids,[8,10,11] polypeptides and proteins,[12] alkaloids,[13,14] antiseptics,[15,16] pepsin in an acid medium[17] and neutral salts.[6,18,19]

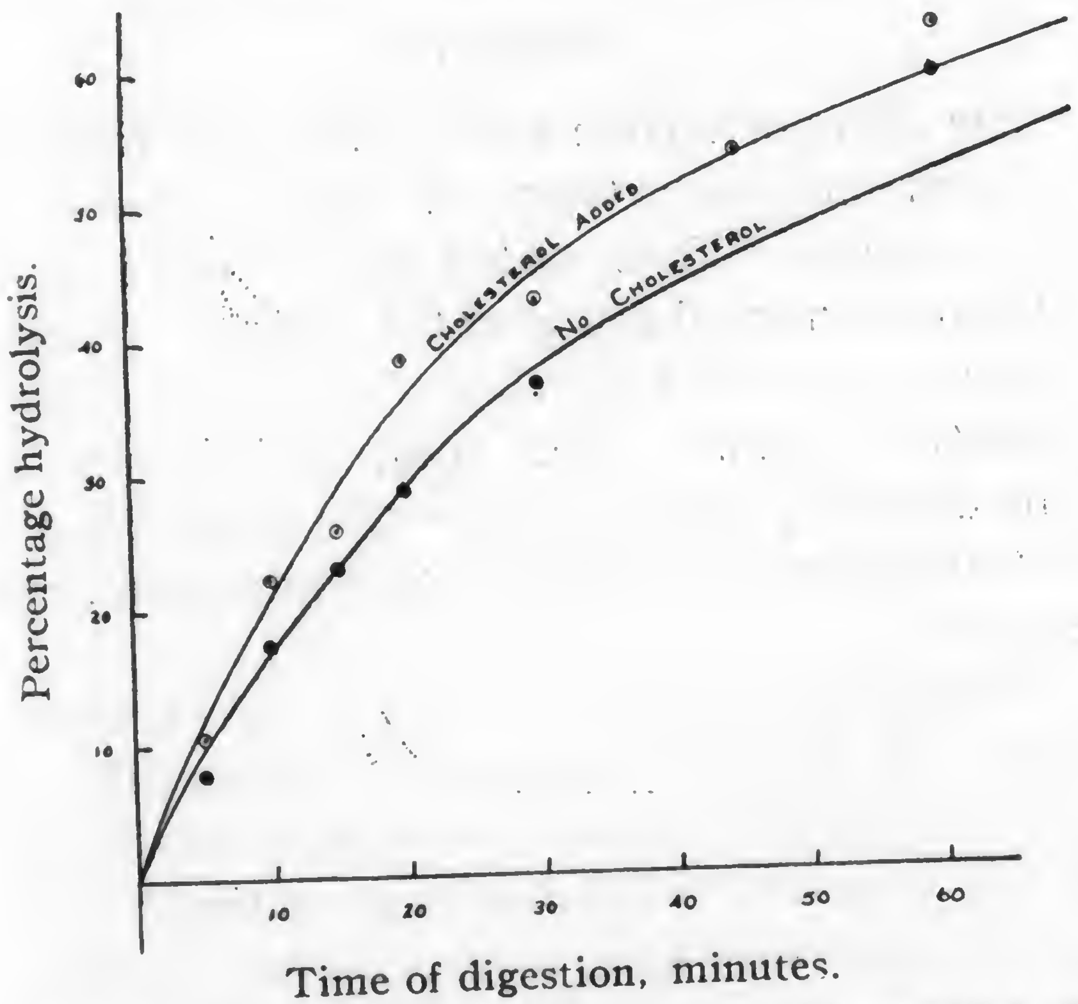

Fig. 1.—The effect of cholesterol on the activity of Ricinus lipase.

Recognizing discrepancies among these reports, the present series of experiments was conducted in an attempt to gain further information on the subject of stimulation or retardation of Ricinus lipase action and with the hope of contributing further evidence as to the nature of the active material in the castor bean.

In connection with the effects of various substances on lipase activity, the opinion apparently prevails that cholesterol retards lipase action. Cholesterol, however, tends to aid the formation of water-in-oil emulsions[20] and it has been observed experimentally[21] that Ricinus lipase appears to be most reactive in an emulsion of this type. Cholesterol should not, *a priori*, affect the lipase as do some materials, *e. g.*, salts as is shown below. The inescapable conclusion was that addition of cholesterol to the digestion mixture should be beneficial to Ricinus lipase action.

Experimental

Ricinus lipase was prepared and its activity estimated by the method of Longenecker and Haley.[21,22] A series of Baker C. P. inorganic salts was added to the digestion mixture and the customary determinations for the extent of hydrolysis of olive oil were made (*cf.* Table I).

The hypothesis regarding the effect of cholesterol was tested by dissolving cholesterol in olive oil and measuring the rate of hydrolysis of the latter in the customary fashion. The data obtained are given graphically in Fig. 1. The concentration of cholesterol (Pfanstiehl C. P.) was 1% with respect to the oil.

Discussion

In no case was there any stimulating affect by the addition of a salt. Salts of cobalt, copper and mercury, even in low concentrations, inhibited lipase action, completely in some cases and to a considerable extent in the others. Salts of calcium, chromium, iron, lead, manganese, magnesium, and zinc likewise were inhibitory but in higher concentrations (when only 25–50 mg. of salt was added, the inhibi-

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[1] Authorized for publication on January 16, 1937, as paper No. 758 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the Pennsylvania State College.

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[3] Nicloux, *Mem. Soc. Biol.*, **56**, 701, 837, 868 (1904).

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[11] Willstätter and Waldschmidt-Leitz, *Z. physiol. Chem.*, **134**, 161 (1924).

[12] Falk, *J. Biol. Chem.*, **96**, 53 (1932).

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[18] Tanaka, *J. Coll. Eng. Imp. Univ. Tokyo*, **5**, 142 (1912).

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[20] Clayton, Text, P. Blakiston's Son and Co., Philadelphia, Pa., 1935.

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[22] Castor beans were furnished by the Baker Castor Oil Co., Newark, N. J.

TABLE I

EFFECT OF INORGANIC SALTS ON RICINUS LIPASE ACTION

In each experiment, 1.00 g. olive oil, 0.050 g. lipase preparation (L. U. = 910) and 0.6 ml. of 0.1 *N* acetic acid containing 100 mg. of the salts indicated were shaken together for three minutes at the start, then let stand at 37–38°.

Salt added	Time of digestion, min. 20	25	40
None (check)	38.8	43.6	53.0
Mercuric acetate	1.2	2.0	2.1
Mercuric chloride	2.6	2.7	2.6
Cupric acetate	4.0	4.1	4.1
Cobalt acetate	10.4	12.0	13.0
Ferric chloride	9.9	10.3	15.5
Zinc chloride	9.6	20.0	23.2
Lead acetate	18.0	20.7	28.0
Lead chloride	21.0	24.0	31.4
Mercurous chloride	22.9	28.1	35.0
Chromium chloride	26.2	27.5	28.5
Nickel chloride	26.9	30.4	38.1
Calcium acetate	28.6	33.9	42.8
Manganese acetate	27.9	33.4	44.8
Manganese chloride	28.5	32.0	40.0
Ferric acetate	32.0	37.6	53.4
Chromium acetate	35.9	42.8	53.0

tion was not so marked in these cases). It is interesting to note that the preparations at this Laboratory invariably contain 7.15–7.25% ash which on qualitative analysis has been shown to contain manganese, magnesium, calcium and iron.[23] These naturally occurring salts have not shown any marked effect on the lipase action except when added in fairly high concentration. These observations support the findings of Willstätter and Waldschmidt-Leitz[11] that manganous salts had no stimulating effect under optimum conditions of activity.

While optimum conditions were maintained in the experiments with cholesterol, a preparation with a lower lipase unitage (L. U. = 285) was used.[24] In this way, cholesterol was shown to have a definite stimulating effect on the rate of Ricinus lipase action. The effect is the more pronounced in the early stages of the hydrolysis, complicating factors[24] probably accounting for the same extent of hydrolysis being reached in each of the three series. It is suggested that this stimulation was due to the formation of a desirable emulsion, water-in-oil. In a separate series of unreported experiments, the effects of other substances chiefly gums known to produce definite types of emulsions were studied. Abnormally low figures were obtained for the percentage hydrolysis in these cases. It is quite possible that these results were due to the emulsifying agents. This problem is receiving further study.

Summary

The effect of certain salts, chiefly chlorides and acetates, on the activity of Ricinus lipase has been studied. Each added salt retarded or inhibited the normal action of the enzyme. Salts of cobalt, copper and mercury showed the greatest inhibiting action. Iron, lead, nickel and zinc salts definitely retarded the action, as to a less extent did the salts of calcium, chromium, and manganese.

Cholesterol has been shown to accelerate the action of Ricinus lipase. This observation is taken to indicate further that a water-in-oil emulsion is desirable for Ricinus lipase action.

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[23] We are indebted to Mr. W. H. Stahl for these analyses.

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STATE COLLEGE, PENNA. RECEIVED JULY 16, 1937

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ENVIRONMENTAL TEMPERATURE AS A FACTOR IN THE PRODUCTION AND IN THE CURE OF RICKETS IN THE RAT [1, 2]

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ONE FIGURE

(Received for publication May 18, 1937)

In the past, nutritional investigators have experienced what appeared to be irregular variations in the antirachitic potency of certain vitamin D preparations. These variations in antirachitic potency have now become recognized as variations in the antirachitic sensitivity of the test animals, and have become more generally apparent since the adoption of the U.S.P. technic for the assay of vitamin D and the subsequent distribution of the reference cod liver oil as a standard of potency. The routine use of definite amounts of this reference standard at monthly intervals offers an excellent opportunity to detect such variations in antirachitic sensitivity. Since these assays are usually carried out according to a carefully standardized procedure, these variations in biological response must result from factors other than those usually considered in the assay technic.

Many investigators have attempted to eliminate these variations in antirachitic sensitivity by observing certain precautions in the feeding and in the care of their breeding colony. Some have considered such factors as size and age of the test

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[2] Presented before the division of biological chemistry at the Chapel Hill meeting of the American Chemical Society, April 12 to 15, 1937.

animals when placed on the rachitogenic diet. Others have considered the problem from the standpoint of environmental factors such as the temperature and the humidity of the environment. In fact, Tourtellotte and Bacon ('35) have presented data from which they have drawn the conclusion that variations in the sensitivity of the rachitic test animals are due to fluctuations in laboratory temperature during the rachitic and test periods. These investigators intimate that high environmental temperature predisposes to short depletion periods and low biological potencies.

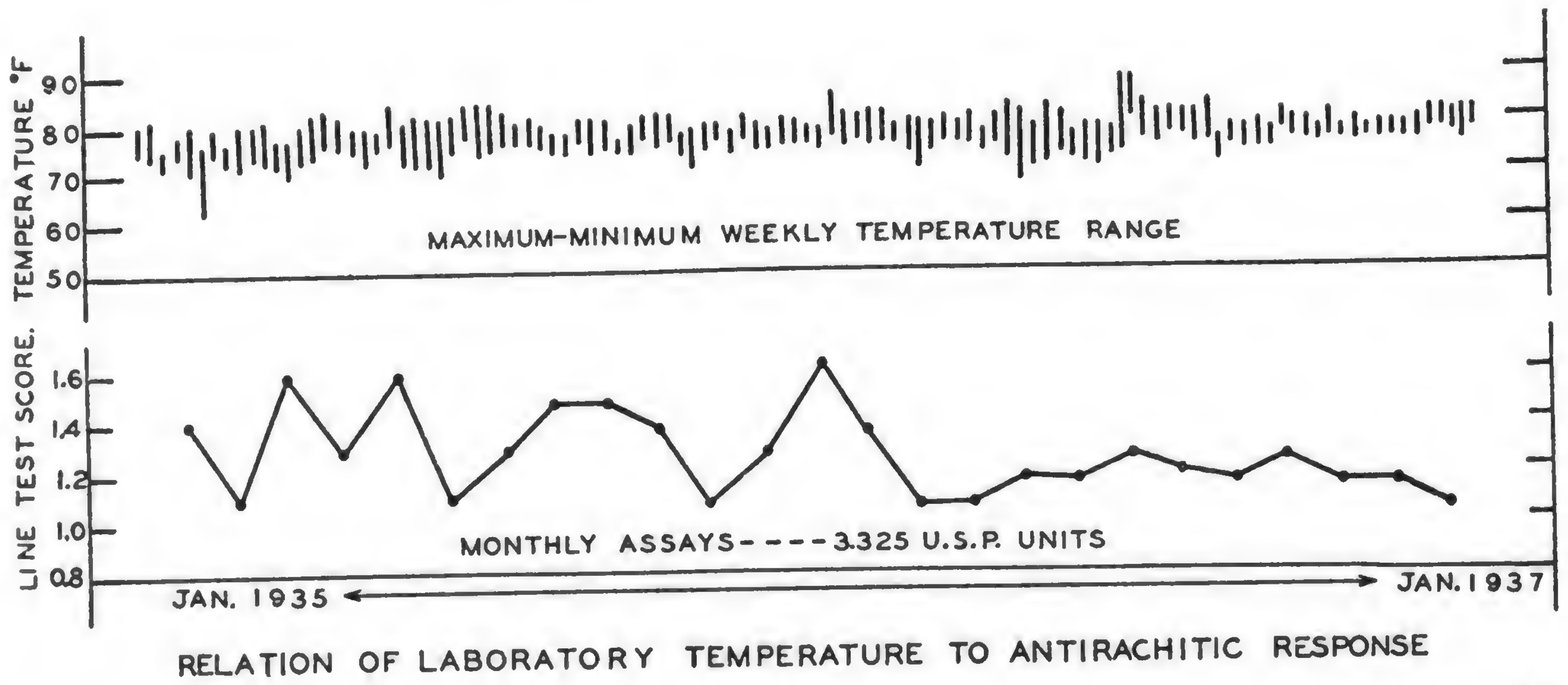

Fig. 1 Showing the relation of the variations in laboratory temperature to the antirachitic potency of the U.S.P. reference cod liver oil as determined by monthly assays during the years of 1935–1936.

If such physical factors as temperature and humidity should be found to be the principal causes of these variations in biological response, this condition might be eliminated once and for all by properly air conditioning the biological laboratory. However, the data available in our laboratory when the above article appeared, failed to show that any uniform relationship existed between the variable sensitivity of our test animals to rickets and the temperature of the experimental laboratory (fig. 1). Realizing, however, that our data had been obtained under a somewhat restricted range of temperatures, we felt that it was not a true index of the influence of temperature on the above condition. It seemed to us that the effect of environmental temperature on the biological response of rachitic animals could be determined most accurately by conducting simultaneous tests at accurately controlled temperatures, using uniform test animals and maintaining all other factors comparable. The following pages describe such a study.

EXPERIMENTAL

Since most biological laboratories are maintained at temperatures approximating 80°F., we decided to maintain our control animals at this temperature. For the extreme, we chose temperatures approximating 70° and 100°F., which we believed to be sufficiently wide to cover the most practical operating conditions. In order to maintain these temperatures under thermostatic control, a special cabinet was constructed of insulating material around one of our regular three-tier laboratory cage units. The three compartments, each containing one tier of cages, were insulated from each other by a double layer of the insulating material. The surfaces of the compartments were reinforced by wooden framework. In order to facilitate the cleaning and the care of the experimental animals, all compartments were provided with a door on each side. Each compartment, containing eight individual cages, was equipped with heating or cooling elements, thermostatic controls, and other necessary equipment to maintain their temperature at the desired level. For the top and the middle compartments, electrical heating elements were used. The temperature of the bottom compartment was maintained by means of a copper coil through which a regulated amount of cold water flowed. This coil was mounted against the top of the compartment and under it was hung a drip-pan to catch and carry away condensed water. When the necessary equipment had been assembled and put through some preliminary tests, it was found that the copper coil in the bottom compartment was not sufficiently large to reduce the temperature of that compartment to 70°F. under all seasonal conditions. It would, however, maintain a temperature of 73 ± 1°F. Because of this fact, the temperature of the middle compartment was adjusted to 84° instead of 80° as previously planned. The experimental equipment, therefore, when placed in operation consisted of three compartments, each of which contained eight individually caged animals that were maintained at temperatures of approximately (±1°F.) 73, 84 and 100°F., respectively.

This investigation consisted of two parts: first, to determine the effect of temperature on the production of rickets, and secondly, to determine the effect of temperature on the recalcification of rachitic rats when fed a definite unitage of vitamin D. In the first of these studies, two series of animals were used, while in the second phase four regular series were found to be necessary and, in addition, several control groups. All test animals were of the same strain, purchased from the same source and were of approximately the same age when placed on experiment. In placing the animals on experiment, unusual care was exercised to select only animals of the same sex and of the same weight for the three compartments. All animals were fed liberal quantities of the rachitogenic diet (Steenbock and Black no. 2965, '25) and were supplied with clean distilled water at all times. In fact, the care and handling was the same as that accorded our regular vitamin D assay animals. Animals used in the recalcification studies were given 2 U.S.P. units of vitamin D on the first and again on the fourth day of the 10-day curative period. The temperatures of the three compartments were carefully recorded each day at a regular hour. The right hind leg of each animal was x-rayed on the eighteenth and the twenty-first (last) day of the depletion period and again on the seventh and the tenth (last) day of the curative period. At the end of the experiments all animals were anesthetized with ether, the blood removed by heart puncture, the tibiae removed for bone ash determinations, and the femora for 'line tests.' The inorganic calcium and the inorganic phosphorus of the serum of the combined blood from all animals in each compartment was determined by the Clark-Collip ('25) modification of the Kramer-Tisdall ('21) method and by the

Youngburg et al. ('30) method. The flesh-free tibiae were dried, extracted in a Soxhlet extractor with ethyl alcohol for a period of 12 hours and again extracted for a like period with sulfuric ether. The extracted bones were again dried, weighed and ashed at a red heat until constant weight resulted.

DATA

The data obtained in these studies have been condensed in tabular form and are presented in the following table. Owing to the close similarity between the x-ray findings among the groups of each series, it was found impossible to express the stages of calcification in terms of a quantitative measure; therefore, no data obtained by this criterion are included. The x-rays were of considerable value, however, in following the progress of decalcification during the depletion period. The relative degrees of decalcification manifested by the negative control animals at the end of the depletion period were evaluated in terms of an arbitrary scale in which conditions approximating mild, moderate and severe rickets were expressed as — 1, — 2 and — 3, respectively. The degrees of recalcification as indicated by the 'line test' were evaluated in the usual manner in which a value of 2 represented a thin but continuous line of darkened granules appearing on the metaphyseal side of the epiphyseal cartilage. Greater or lesser degrees of recalcification were designated accordingly. The following data represent the average values of all animals of the respective groups.

DISCUSSION

Since the above experiments were carried out during a period extending from late winter to early fall, the test animals used should have been fairly representative of those commonly employed in biological assays so far as the seasons of the year are concerned. With every experimental series, unusual efforts were made to select only vigorous animals of the same sex and of the same weight for each of

TABLE 1

Showing the average values obtained from the various groups of animals during the investigation. These values have been tabulated in the order of increased environmental temperature and also according to the three stages of calcification represented

GROUP NO.	NUMBER OF ANIMALS	AVERAGE TEMPERATURE OF COMPARTMENT	AVERAGE INITIAL WEIGHT	DEPLETION PERIOD	CURATIVE PERIOD	UNITS VITAMIN D FED	AVERAGE FINAL WEIGHT	AVERAGE DAILY FOOD INTAKE	AVERAGE 'LINE TEST' SCORE	SERUM		AVERAGE BONE ASH
										Ca per 100 ml.	P per 100 ml.	
Negative controls—rachitogenic diet without supplement												
		°*F.*	*gm.*	*days*	*days*	*U.S.P.*	*gm.*	*gm.*	*− or +*	*mg.*	*mg.*	*%*
1-A	8	74	45	21	0	0	65	5.7	−1.9	10.4	3.3	36.6
2-A	6	72	45	21	0	0	61	5.1	−1.8	11.1	4.5	36.7
1-B	8	84	45	21	0	0	62	5.0	−1.8	12.3	3.5	38.8
2-B	8	84	44	21	0	0	60	4.6	−2.0	11.3	4.3	38.4
7-B	8	84	43	31	0	0	70	4.9	−2.5	10.1	2.9	29.8
1-C	7	101	45	21	0	0	58	3.6	−2.0	10.8	3.2	37.7
2-C	4	100	43	21	0	0	56	3.4	−2.0	12.3	4.6	40.3
Experimental—rachitogenic diet plus vitamin D supplement												
3-A	5	73	43	21	10	4	67	6.5	+1.6	9.5	6.3	32.8
4-A	8	72	45	21	10	4	67	6.3	+1.5	10.7	9.2	43.5
5-A	7	73	46	21	10	4	70	5.8	+1.9	10.7	5.2	34.3
6-A[1]	6	72	44	21	10	4	64	5.9	+1.8	12.7	7.7	36.4
3-B	8	84	45	21	10	4	66	5.6	+1.3	12.6	6.7	34.5
4-B	7	85	45	21	10	4	68	5.5	+1.3	10.8	8.4	41.2
5-B	8	84	46	21	10	4	72	5.0	+1.9	12.8	4.9	32.7
6-B[1]	8	84	44	21	10	4	61	4.7	+1.5	12.5	5.0	36.0
3-C	(All animals over heated)											
4-C	7	100	45	21	10	4	66	3.9	+1.7	10.5	6.8	43.6
5-C	6	101	46	21	10	4	61	3.1	+1.2	11.7	4.9	34.7
6-C[1]	6	100	44	21	10	4	56	3.2	+1.7	12.4	6.2	36.7
Positive controls—receiving breeding colony diet												
8-B	8	84	44	2 days at 84°F.			48	6.9	Normal	11.3	10.1	51.7
9-B	6	84	43	6 days at 84°F.			60	8.2	Normal	11.7	9.3	52.2
10-B	8	84	45	10 days at 84°F.			71	9.6	Normal	11.4	9.4	53.5

[1] All animals composing these groups were females. All other animals used in the investigation were males.

the three compartments. In fact, the method of selection was so exacting that in some series the individual weights of all animals of the three compartments, when placed on the rachitogenic diet, did not differ by more than 3 gm. All animals used were males except those of the sixth series, which were all females.

For the sake of reference, the three experimental compartments were designated from bottom to top as compartments A, B and C, respectively. As previously stated, the approximate temperatures at which these compartments were maintained were 73, 84 and 100°F., respectively. The various series of animals used were designated numerically in chronological order. A reference to a particular group, for example group 5-B, denotes, therefore, the fifth group of animals maintained in compartment B at a temperature approximating 84°F. Other groups will be similarly designated.

The relation of environmental temperature to the daily food intake of the various test animals became distinctly evident during the first series of studies and remained equally evident throughout the entire investigation. As was to be expected, those animals which were subjected to the lower temperatures consumed greater quantities of the rachitogenic diet. In fact, at the end of the depletion period those groups of animals which were maintained at temperatures of 84 and 73° had consumed, on an average, 13 and 31% more food, respectively, than had similar animals maintained at 100°F. When the experiments were continued over a 10-day curative period, these differences in food consumption increased to 50 and 82%, respectively. The average increase in body weight was also greater at the lower temperatures. This would indicate that, under the conditions of the investigation, the rat's requirement for food was not restricted to that required for energy production plus the additional amount required to produce a uniform rate of growth. The relative amounts of food required to produce a unit gain in body weight at the temperatures studied were: 5.9, 5.1 and

4.5 gm., respectively, at the end of the depletion period and 8.7, 7.5 and 6.6 gm., respectively, at the end of the curative period.

The degree of rickets manifested at the end of the depletion period, as indicated by the line test, showed no significant differences that might be in any way attributed to differences in environmental temperature. There was a very slight indication that more severe rickets were produced at the higher temperature, but it appears doubtful if the method of scoring the rachitic condition was sufficiently sensitive to make such differences significant.

The line test score as determined at the end of the curative period did not indicate that environmental temperature is an important consideration in recalcification in the rat. The average values of the scores do indicate slightly greater recalcification at the lower temperatures, but differences among these values are less than differences among line test values obtained from different groups of animals maintained at the same temperature and otherwise treated identically.

Those animals which had received the rachitogenic diet during a 21-day depletion period likewise failed to show that environmental temperature had any definite effect on serum calcium, serum phosphorus or bone ash. Here again groups of animals of different series which had been maintained at the same environmental temperature showed as much variation in these values as did groups of animals of the same series which had been maintained at different temperatures. At the end of the curative period, however, there were indications that a higher environmental temperature resulted in a higher serum calcium and a lower serum phosphorus. Here again group differences were marked and it appears doubtful if the apparent temperature effects were significant.

A point which appears worthy of mention concerns the relative mortality among the groups of animals maintained at the different temperatures. It may also be well to state that the third group of animals (group 3-C) maintained at the higher temperature was accidentally subjected to an increased temperature (108°F.) during one night of the second week of the experiment, due to the improper functioning of the thermostat. As a result of this increased temperature, a number of the animals died during the night and others were so severely weakened that the entire group was discarded. Other than this group, the total mortalities numbered a total of twenty-seven, of which eight occurred at the lowest temperature, one at the medium temperature and eighteen at the highest temperature. Nothing was observed, however, that would indicate that the higher mortality was in any way related to a more severe rachitic condition.

We were somewhat surprised to find that those animals which had been maintained at the lowest temperature and which had consumed the greatest amount of food, and in consequence had made the greatest gain in body weight, did not show a significant difference in the degree of rickets manifested. In our regular vitamin D assays, where all animals are maintained at a comparable temperature, those animals which make the greatest increase in body weight invariably show the highest degree of rickets.

A general consideration of these data leads us to conclude that environmental temperatures, within the ranges studied, are rather unimportant considerations in either the production or the cure of rickets in the rat. On the other hand, the data do indicate that the history of the test animal previous to being placed on the rachitogenic diet is a worth while consideration. This is well illustrated by the animals comprising the fourth series (groups 4-A, 4-B and 4-C) which showed considerably higher serum phosphorus and bone ash values at all temperatures than did the animals of any of the other series. Such variations in test animals appear to be independent of the body weight when placed on experiment. It is highly probable that this can be at least partially attributed to unrecognized variations in the breeding colony diet.

SUMMARY

A series of studies have been made concerning the effect of environmental temperature on the production and on the cure of rickets in the rat. Comparable experiments were carried out at temperatures of 73, 84 and 100°F., using a uniform rachitogenic diet and carefully selected test animals. The stages of calcification were measured by means of x-rays, line test, bone ash, and serum calcium and phosphorus.

The data obtained in these studies indicate that environmental temperature is not an important consideration in either the production or the cure of rickets in the rat. In fact, undetectable differences in different groups of rats when placed on the rachitogenic diet led to greater variations in experimental results than did a variation of approximately 28°F. in environmental temperature.

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RETARDING
HAMS ANI

By PROF. P. THOS.

THE susceptibility to ran
such fatty meat products
and bacon, and particular
bacon, is well recognized. Ra
these products first takes the
loss of fresh flavor and then
objectionably until the meat

PREVENTING RANCIDITY

coated with Avenex No. 4. Althoug
this was no adequate test from whic
to draw conclusions, evidence secure
in other tests indicates that for dry cu
ing pork at relatively high uncontrolle
temperatures, Avenex No. 4 coated ove
the hams at the last rubbing will assi
materially in deterring rancidity.

Smoked Bacon in Avenex No. 3

Cured and smoked bacon slabs wer
packed in a box and as each slab wa
packed it was sprinkled with a coatin
of Avenex No. 3, using from 1 to 1$^1/$
ounces of the Avenex to each slab. Th
entire group of bacons was then cover
with the Avenex and stored at roo
temperature of 70° F. for two month
At the end of this period a sample wa
removed and cooked, and it tested ex
cellent for color and flavor. Th
Avenex was easily brushed off and di
not spoil the appearance of the bacon
Samples obtained from the untreate
bacon slabs were decidedly more ranci

Avenol Sprayed on Bacon

In this test two slabs were spraye
with just sufficient Avenol No. 1 t

SUMMARY

A series of studies have been made concerning the effect of environmental temperature on the production and on the cure of rickets in the rat. Comparable experiments were carried out at temperatures of 73, 84 and 100°F., using a uniform rachitogenic diet and carefully selected test animals. The stages of calcification were measured by means of x-rays, line test, bone ash, and serum calcium and phosphorus.

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RETARDING RANCIDITY IN HAMS AND BACON

By PROF. P. THOS. ZIEGLER and DR. R. C. MILLER*

THE susceptibility to rancidity of such fatty meat products as hams and bacon, and particularly sliced bacon, is well recognized. Rancidity of these products first takes the form of loss of fresh flavor and then develops objectionably until the meats actually become inedible. Even with refrigeration and rapidity of distribution, this problem remains, for it is in many cases impossible to control time and conditions from production to consumption sufficiently to prevent the development of rancidity at least to some degree.

Work has already been done confirming the rancidity-retarding properties of oat flour known as Avenex for such products as ice cream and frozen cream(1), potato chips(2, 3), and cured mackerel(4). More recent work supplementing the patent literature (5, 6) has indicated the marked increase in the stability of the fat of bacon when using oat flour as part of the curing ingredients and for dusting over sliced bacon.(7)

In an effort to confirm these findings, studies were undertaken to determine the inhibitory properties of oat flour and oat oil (the hexane extract of oat flour). Samples of whole pulverized oats known as Avenex No. 3, decorticated oat flour known as Avenex No. 4, and oat oil known as Avenol No. 1 were supplied by the Quaker Oats Co. for this work.

Avenex on Bacon

Thoroughly chilled bacon slabs were dry sugar cured at a controlled temperature of 40° F. Twelve days later the bacon slabs were coated with Avenex No. 3 and a similar number were put up with Avenex No. 4 applying in each case approximately 1½ ounces of Avenex to an average slab of bacon. The same number of bacon slabs were cured without treatment with the Avenex. All bacons were held for 6 weeks, at the end of which time they were soaked, smoked, and tested. The uncoated bacons were slightly rancid, whereas the bacons treated with both Avenex No. 3 and Avenex No. 4 were found to be still sweet.

This would indicate that the Avenex treatment of heavy bellies and fat backs which are dry salted might aid materially in holding down rancidity.

Avenex on Hams

Hams that are cured under uncontrolled conditions (not under refrigeration) are very often lost through ham souring or the development of rancidity. The chief precaution against ham souring is immediate and thorough chilling. In this test, thoroughly chilled hams were dry sugar cured at a room temperature of 65° F., which is 20° above what a dry curing room temperature should be. It was an impractical temperature but a rigorous test for rancidity. The dry sugar cure was applied to the ham in two rubbings, the second rubbing being applied three days later. At the time of the second rubbing, one ham was coated with Avenex No. 3, one with Avenex No. 4, and the third was left to cure naturally. They were placed on a shelf and left to cure for thirty days, which was approximately seven days per inch of ham. At the end of this period they were soaked for two hours and the Avenex was then washed from the ham by the application of a strong water spray. The hams were smoked and then chilled for 24 hours, when a cooking as well as a rancidity test was made.

The ham coated with Avenex No. 3 had soured, due either to the exclusion of too much air or the condition of the ham itself. The ham that was uncoated and the ham coated with Avenex No. 4 were in good condition, with the exception that the uncoated ham was decidedly more rancid than the ham

MEAT Magazine believes that it is one of its functions to keep the packing industry informed on current trends, new methods and new products. Even though these products or methods are still unproved, the packer will want to keep abreast of current trends of research. in meat processing.

This article describes the results of tests which have been made on the use of Avenex (an oat flour product) for reducing rancidity in hams and bacon. This process has already proved very effective in retarding rancidity in packaged lard and butter.—Ed.

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* *The tests reported in this article were conducted for the Musher Foundation.—Ed.*

PREVENTING RANCIDITY

coated with Avenex No. 4. Although this was no adequate test from which to draw conclusions, evidence secured in other tests indicates that for dry curing pork at relatively high uncontrolled temperatures, Avenex No. 4 coated over the hams at the last rubbing will assist materially in deterring rancidity.

Smoked Bacon in Avenex No. 3

Cured and smoked bacon slabs were packed in a box and as each slab was packed it was sprinkled with a coating of Avenex No. 3, using from 1 to 1½ ounces of the Avenex to each slab. The entire group of bacons was then covered with the Avenex and stored at room temperature of 70° F. for two months. At the end of this period a sample was removed and cooked, and it tested excellent for color and flavor. The Avenex was easily brushed off and did not spoil the appearance of the bacon. Samples obtained from the untreated bacon slabs were decidedly more rancid.

Avenol Sprayed on Bacon

In this test two slabs were sprayed with just sufficient Avenol No. 1 to make them sparkle but not run or drip. Two other bacon slabs were left untreated. At the end of a 5 months storage period at room temperature of 65° F. the peroxide value of all the slabs of bacon was about the same, none running over 2.17, but the organoleptic tests showed that the sprayed bacon slabs were far more desirable in flavor and very much less rancid than the untreated bacons.

Samples of sliced bacon were also sprayed with Avenol No. 1 and wrapped in bulk in regular parchment paper. At the end of four months the treated samples were in excellent shape as to color and taste, whereas the untreated sliced bacon samples were yellow and distinctly rancid. The peroxide values confirmed the organoleptic observations.

From these experiments conducted using oat products it is evident that both oat flour and oat oil may be of distinct value in retarding the development of rancidity in cured hams and bacon.

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THE CARBON METABOLISM OF FUSARIUM LYCOPERSICI ON GLUCOSE

BY

GORDON H. PRITHAM AND ARTHUR K. ANDERSON
(Contribution from Pennsylvania Agricultural Experiment Station)

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THE CARBON METABOLISM OF FUSARIUM LYCOPERSICI ON GLUCOSE[1]

By GORDON H. PRITHAM, *graduate scholar*, and ARTHUR K. ANDERSON, *professor of physiological chemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

This paper reports the results of the fourth in a series of studies begun by Anderson in 1924, when he published on the biochemistry of *Fusarium lini* (*1*).[2] The second and third papers were from this laboratory, one in 1933, the other in 1934 (*2, 3*); both deal with *Fusarium oxysporum*. The present investigation was undertaken to obtain data on a third *Fusarium*, which would provide a basis of comparison with the previous studies.

REVIEW OF LITERATURE

One of the most important investigations of tomato wilt is that of White (*17*), who in 1927 made a study of 24 strains of *Fusarium lycopersici* with respect to pathogenicity, influence of the hydrogen-ion concentration of the medium on the growth of the organism, products of metabolism on a glucose medium, and the toxicity of these products to tomato plants.

In a study of the hydrogen-ion concentration of the soil as related to the *Fusarium* wilt of tomato seedlings, Sherwood (*14*) found that acid soils favor wilting, but that there is no limiting degree of acidity or of alkalinity at which the disease will not develop. May (*8*) found no correlation between pH or titratable acidity of five varieties of tomatoes and resistance to *Fusarium* wilt at any stage of growth. Scott (*13*) obtained maximum growth of *Fusarium lycopersici* at pH values between 4.5 and 5.3, the organism tending to change the pH of all solutions toward a constant value of 6.0.

In a recent study of the metabolism of *Fusarium lycopersici*, Luz (*7*) observed four phases in the changes in hydrogen-ion concentration of the medium brought about by the growth of the fungus. He ascribes these changes to the formation of organic acids and to the preferential absorption of ions by the fungus. He also found that, as soon as the glucose is exhausted, the organism very rapidly utilizes the ethyl alcohol which it produces.

A considerable amount of work has been done on the biochemistry of *Fusarium lini* by Anderson (*1*), Letcher and Willaman (*6*), White and Willaman (*15, 16*), and Reynolds (*11*). This work, together with

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[1] Received for publication Mar. 12, 1937; issued February 1938. Paper No. 791 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This paper is part of a thesis presented by Gordon H. Pritham to the faculty of the Graduate School of The Pennsylvania State College, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Reference is made by number (italic) to Literature Cited, p. 948.

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that of Birkinshaw, Charles, Raistrick, and Stoyle (*5*) on various species of *Fusarium*, is reviewed elsewhere (*3*). Reynolds (*10, 12*) demonstrated in resistant flax plants a cyanogenetic glucoside, linamarin, which inhibited the growth of *F. lini*, and he postulated that the fungus may release enough hydrocyanic acid from a resistant plant to inhibit its own growth.

Anderson, Everitt, and Adams (*3*) repeated much of Anderson's earlier work, using *Fusarium oxysporum* instead of *F. lini*. They found that the metabolism of the two organisms on glucose is very similar, the principal difference being that *F. oxysporum* utilized very little of the ethyl alcohol which it produced, while *F. lini* used it to a marked degree. Anderson and Emmart (*2*) studied the effect of adding certain amino acids to the culture medium on the rate of carbon dioxide production by *F. oxysporum*. The amino acid studied had little effect on the metabolic rate.

GENERAL METHODS AND MATERIALS

Source of Fusarium lycopersici culture.—The culture of the organism used in this investigation was obtained from Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station. It was isolated from Maryland tomatoes and was known as culture M. From this culture a monosporous culture was prepared by the authors. Transfers from this culture were used in the present study.

Preparation of the medium.—The medium used in this work had the following composition:

Ammonium nitrate	1. 00 g.
Magnesium sulphate	0. 25 g.
Monopotassium phosphate	0. 50 g.
Distilled water to make	1, 000 ml

To this stock solution, sufficient glucose was added to make an approximately 2-percent solution of glucose. The exact quantity of glucose in each lot of medium was determined by the Folin-Wu (*7*) method after autoclave sterilization.

Hydrogen-ion concentration.—The pH value of all solutions was determined electrometrically. The quinhydrone electrode was used in all cases except in the study of the influence of the hydrogen-ion concentration on the growth of the fungus, when the Bailey hydrogen electrode was employed.

Determination of combustible gases.—Since a low percentage recovery of carbon was obtained in preliminary work, where the methods were the same as those used in earlier work on *Fusarium lini* (*1*) and *F. oxysporum* (*3*), it was thought that possibly the fungus might produce some gases or volatile compounds which were not absorbed during aeration of the flasks for carbon dioxide. Consequently, a combustion tube, partially filled with ignited lead chromate and wire-form cupric oxide, as recommended by Pregl (*9*), and another barium hydroxide tower were added to the aeration train, the gases being passed through the hot combustion tube and into the second barium hydroxide tower after going through the first barium hydroxide tower. A significant amount of carbon was recovered by this method. That this was not merely some carbon dioxide which had escaped absorption in the first tower was shown by interposing another barium hydroxide tower between the first one and the combustion tube. Very little carbon dioxide was obtained in this tower.

Determination of nitrogen in the mycelium.—Since Anderson and Schutte (*4*) have shown that, in the absence of chlorides, the residue from the determination of carbon by the wet combustion method can be used satisfactorily for the determination of nitrogen by the Kjeldahl method, the residue from each carbon determination after digestion was made strongly alkaline with saturated sodium hydroxide solution, the ammonia being distilled and titrated as in the usual Kjeldahl method.

The methods for the preparation and inoculation of the culture flasks, the determination of carbon dioxide, ethyl alcohol, residual glucose, organic acids, and carbon in the mycelium are essentially the same as those used in previous work (*1, 3*).

THE REACTION OF THE MEDIUM IN RELATION TO GROWTH

OPTIMUM, MAXIMUM, AND MINIMUM pH

The technique used in studying the relation of the reaction of the medium to growth of *Fusarium lycopersici* was similar to that employed by Anderson (*1*) in his work with *F. lini*. The results are presented in figure 1. It is evident that *F. lycopersici* is very tolerant to extremes of acidity and alkalinity, the minimum pH value for growth being 1.88 and the maximum 11.06. These results correspond well with those obtained by White (*17*) with *F. lycopersici* and with those of Anderson (*1*) with *F. lini*. Luz (*7*), however, obtained no growth of *F. lycopersici* at pH values above 8.10. The lowest pH that he tried was 3.25, at which value the fungus grew well.

The range for good growth is also very wide, extending from about pH 2.0 to pH 9.5, the optimum being pH 4.1. Scott (*13*) and White (*17*) obtained almost identical results with *F. lycopersici*, but observed two maxima in the growth curves at pH 4.0 to pH 5.3 and above pH 7, with a minimum between pH 5 and 7. Luz (*7*) observed maximum growth at pH 5.7. The results of the present study also agree well with those found for *F. lini* (*1*).

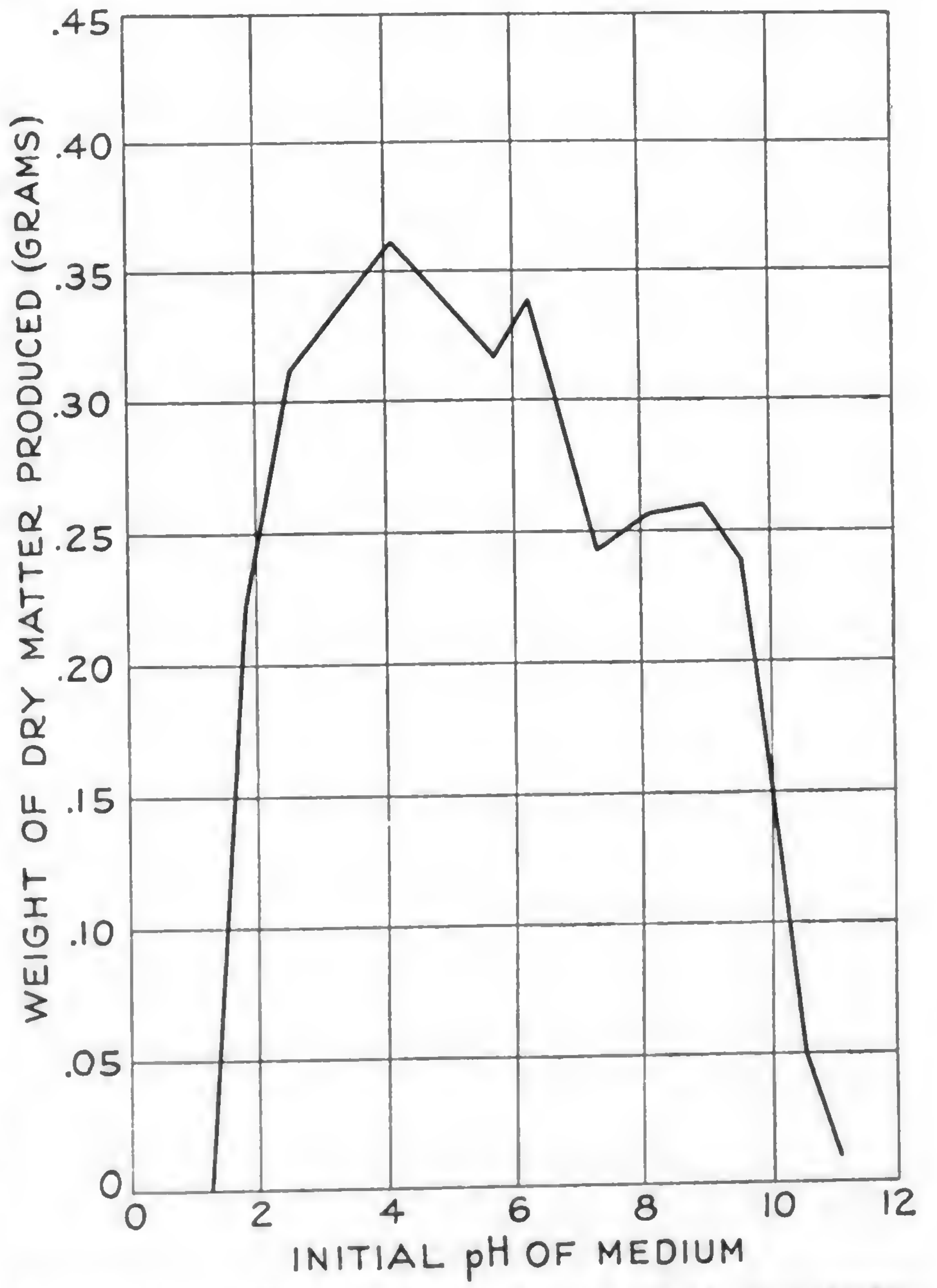

FIGURE 1.—Growth of *Fusarium lycopersici* at various hydrogen-ion concentrations.

CHANGE IN pH DURING GROWTH

Figure 2 shows graphically the change in the reaction of the medium with time, both with and without the growth of the fungus. Perhaps the most striking effect is the marked tendency of the organism, within the range of vigorous growth, to change the reaction of the medium toward the optimum value. This effect is especially notable on the acid side, the final pH values of five cultures that had initial reactions of from 1.88 to 6.28 varying only from 4.25 to 4.95—values that are well within the optimum pH range. That these changes are due to the growth of the fungus and not to independent changes in the reaction of the medium itself is shown by the fact that the change in the reaction of the uninoculated medium tends to be constant. The final pH of the blanks bears an approximately constant ratio to the initial pH, except in the strongly alkaline solutions, where there is evidently considerable absorption of carbon dioxide from the air. Although the change in the reaction of the inoculated medium is not so striking on the alkaline side as on the acid side of neutrality, the organism shows a decided tendency to increase the hydrogen-ion concentration toward the optimum. Part of this increase evidently is due to absorption of carbon dioxide from the air, but here again the change in the inoculated flasks is greater than in the uninoculated ones, indicating that the fungus itself changes the reaction of the medium. These results are similar to those obtained by Scott (*13*) with *Fusarium lycopersici*, and by Anderson (*1*) with *F. lini*.

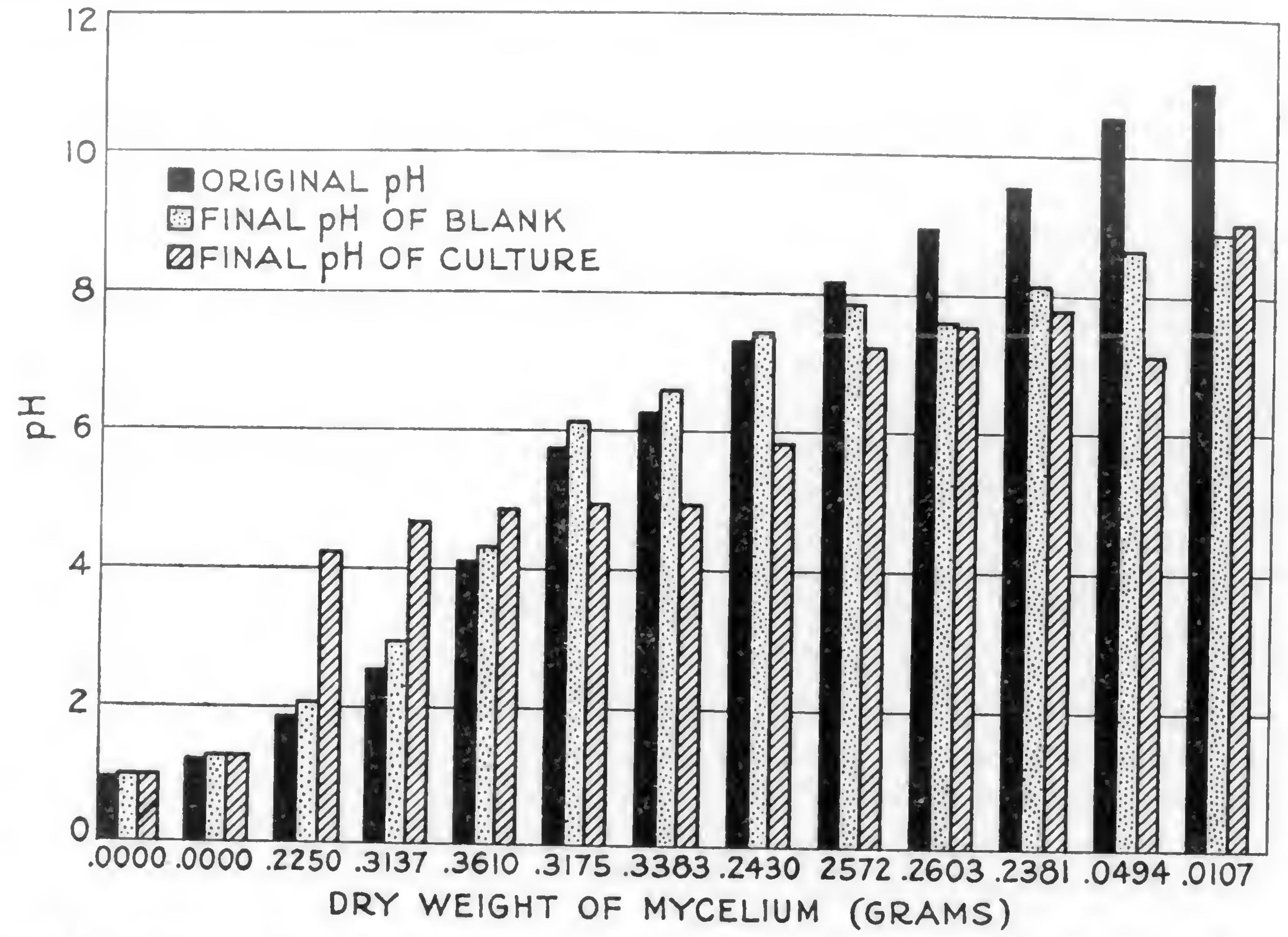

Figure 2.—Changes in hydrogen-ion concentration of the medium during growth of cultures shown in figure 1.

In studies of the carbon metabolism on glucose, the change in pH was followed at intervals over a considerable period of growth. In all, four studies were made, and in every case the pH value of the medium was increased to about 7.0. There was no initial decrease, as Luz (*7*) observed in his work with *Fusarium lycopersici*, and as Anderson (*1*) found with *F. lini*. The pH value of an 8-months-old culture of *F. lycopersici*, which was used in testing for organic acids, was 7.3, showing that the fungus will change the reaction of the medium from acid to alkaline if allowed to grow long enough. Luz (*7*) ascribes the increase in pH to 7.5, as observed by him, to the preferential absorption of nitrate ions from ammonium nitrate in the medium, with the concurrent liberation of ammonium ions.

CARBON METABOLISM ON GLUCOSE

Although four studies (series) were made, the results of only the last will be presented in detail as it is representative of the other three (table 1 and fig. 3). Since in the first series the total recovery of the original carbon decreased to less than 50 percent as growth progressed, it was thought that the fungus might produce some compounds other than the ones being determined. Accordingly, two new methods were introduced in the subsequent series. It was suggested that some of the undetermined carbon might be in the form of gaseous or volatile compounds which would be lost during the aeration of the cultures for carbon dioxide. To test this hypothesis, a combustion tube and a barium hydroxide tower were introduced into the aeration train. By means of this technique, the results of which are given as "Combustion" in table 1 and figure 3, an amount of carbon representing about 2 percent of the original carbon was obtained, thus showing that a small amount of volatile organic compounds is produced by *Fusarium lycopersici*.

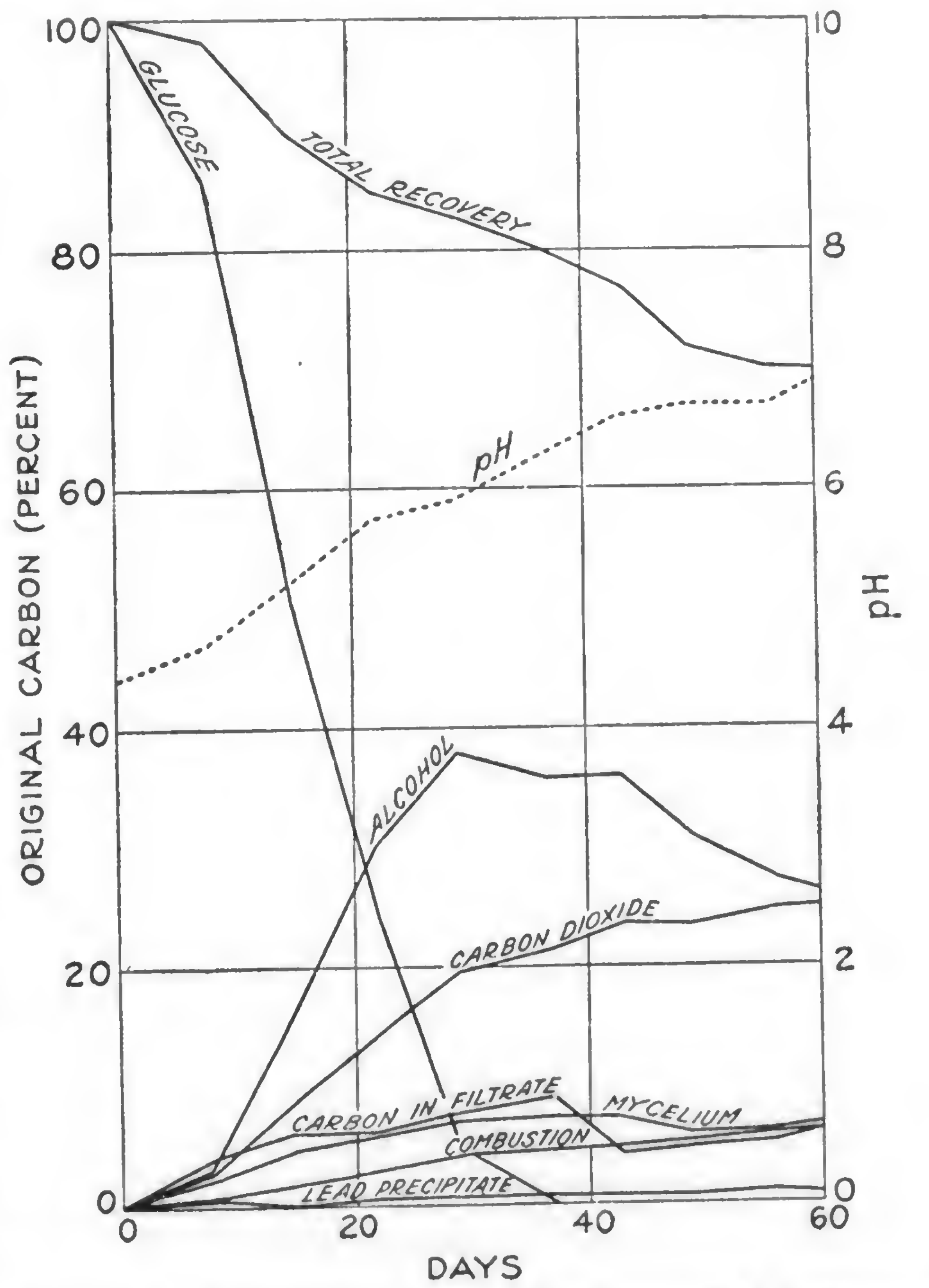

Figure 3.—Distribution of metabolic products of *Fusarium lycopersici* when grown on glucose. See table 1.

TABLE 1.—*Distribution of metabolic products of Fusarium lycopersici on a glucose medium at various stages of growth*

Age of culture (days)	Mycelium					Carbon dioxide		Ethyl alcohol	
	Weight	Weight of carbon	Carbon	Carbon as related to initial carbon [1]	Nitrogen	Weight of carbon	Carbon as related to initial carbon [1]	Carbon in 300 ml	Carbon as related to initial carbon [1]
	Gram	*Gram*	*Percent*	*Percent*	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*
0									
8	0.120	0.081	45.88	2.31	3.62	0.068	2.97	0.075	3.20
15	.230	.110	47.79	4.70	3.22	.213	9.08	.374	15.96
22	.307	.140	45.62	5.98	3.33	.341	14.55	.712	30.44
29	.347	.169	48.56	7.20	4.27	.446	19.55	.890	38.01
37	.383	.177	46.26	7.58	4.03	.502	21.46	.825	35.76
43	.385	.176	45.91	7.54	4.00	.549	23.45	.841	35.96
49	.341	.143	41.63	6.12	3.68	.547	23.36	.706	30.73
56	.366	.147	40.00	6.26	3.38	.574	24.55	.644	27.32
60	.365	.156	42.67	6.65	3.62	.575	24.58	.612	26.11

Age of culture (days)	Glucose		Lead precipitate		Combustion		Residual carbon in filtrate		Total recovery of carbon as related to initial carbon[1]	pH
	Carbon in 300 ml	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]		
	Gram	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*	*Percent*	
0	2.34	100.0							100.0	4.4
8	2.01	85.8	0.010	0.42	0.024	0.70	0.06	4.00	98.0	4.7
15	1.19	50.9	.003	.12	.044	1.88	.15	6.20	90.0	5.2
22	.57	24.2	0.008	.40	.071	3.03	.15	6.20	85.0	5.8
29	.12	5.0	.020	.80	.101	4.30	.17	7.90	83.0	5.9
37	.01	.3	.023	1.00	.112	4.80	.20	8.70	80.0	6.3
43	.00	.0	.023	1.00	.119	5.07	.11	4.48	77.0	6.6
49	.00	.0	.021	.87	.128	5.35	.12	4.70	72.0	6.7
56	.00	.0	.028	1.15	.141	6.04	.13	5.30	70.0	6.7
60	.00	.0	.023	1.00	.143	6.10	.14	6.00	70.0	6.9

[1] Expressed as percentage of the carbon in the glucose originally present.

As a further check on the recovery of carbon, an aliquot of the filtrate from the original cultures was analyzed for total carbon by the wet combustion method. The sum of the carbon in the alcohol, residual glucose, and lead precipitate was then subtracted from this total carbon, giving the undetermined, or residual carbon in the filtrate. A maximum of 8.7 percent of the original carbon could be accounted for in this way, indicating that a significant amount of some organic compounds, other than ethyl alcohol, which are not precipitated by lead acetate, is formed by the organism from glucose. The curve for the total recovery of carbon in figure 3 includes both the residual carbon and the carbon obtained by combustion.

Table 1 and figure 3 show that ethyl alcohol and carbon dioxide are the main products of the metabolism of the organism on glucose, the alcohol being produced slowly at first and then more rapidly until the glucose has been exhausted, when it decreases fairly rapidly. Since carbon dioxide production contines to increase after the consumption of the glucose, it is evident that *Fusarium lycopersici* can utilize ethyl alcohol for energy and for growth. These facts are in accord with the results obtained by Anderson (*1*) with *F. lini*, except that the decrease in the alcohol, as well as the rate of metabolism for the entire series, is not as rapid as he found it to be. Anderson, Everitt, and Adams (*3*) found that *F. oxysporum* was able to utilize ethyl alcohol as a source of carbon to only a slight extent. In this connection the recent work of Luz (*7*) should be mentioned. He found that *F. lycopersici*, when grown on a medium containing 5 percent of glucose, produced alcohol rapidly until the glucose was exhausted on the eighteenth day of growth, when the alcohol was used at a rapid rate until it was finally exhausted on the thirty-fifth day of growth. Since, in the present study, a considerable amount of alcohol was present in the medium after 60 days' growth, it is obvious that the rate of metabolism of the strain of *F. lycopersici* used by him was much greater than that of the strain employed in this investigation. However, his mineral nutrient solution was 10 times as concentrated as the one used in the present work.

The lead precipitate is significant because it is nearly constant, although representing only a small fraction of the original carbon. Evidently *Fusarium lycopersici* produces only small amounts of organic acids, the lead salts of which are insoluble. Anderson (*1*) also found that only small amounts of these acids were produced by *F. lini*.

The residual carbon in the filtrate increases rather rapidly until the glucose is practically exhausted, and then decreases. Since this decrease is at a somewhat more rapid rate than the corresponding decrease in alcohol and in the total carbon in the filtrate, it is evident that the fungus utilizes this carbon more easily than it does ethyl alcohol. As old cultures have an esterlike odor resembling that of apricots, it is possible that this residual carbon is represented by some organic acids which were not determined by any of the methods used.

The amount of volatile compounds determined by combustion shows a gradual increase during the entire growth period. The highest value was obtained in the last series at 60 days, when 6.1 percent of the original carbon was in this form. At least a part of this carbon may be in the form of volatile acids, since in subsequent experiments a small amount of these acids was found in old cultures of the organism.

The changes in the pH of the medium with growth are practically uniform in all series and the final pH is nearly the same in all series, being well within the optimum range for growth. Luz (*7*) found that he could divide the growth of *Fusarium lycopersici* into four stages on the basis of the pH changes during growth, but no such periods, or "breaks," in the pH curves were obtained in this work. Anderson (*1*) observed that *F. lini* first decreased the pH of the medium and then gradually increased it, the final value depending on the initial pH of the medium.

QUALITATIVE TESTS FOR ORGANIC ACIDS

The filtrate from an 8-months-old culture of *Fusarium lycopersici* was distilled from acid solution, and the distillate titrated with standard alkali. An amount of volatile acid equivalent to about 4.0 ml of normal acid, or 0.24 g of acetic acid, was obtained. The residue from the distillation was tested for oxalic acid by the usual precipitation as calcium oxalate. Since there was obtained a white precipitate which decolorized potassium permanganate in sulphuric acid solution, it was concluded that oxalic acid was formed in small amounts, thus confirming the findings of Luz (*7*). A doubtful test for tartaric acid was also obtained.

COMPOSITION OF MYCELIUM

The data for the percentage of carbon and of nitrogen in the mycelium, calculated on the dry weight of mycelium, are given in the column headed "Mycelium" in table 1. There is a tendency for the carbon content of the mycelium to decrease with age, a change exactly opposite to that found for *Fusarium lini* (*1*) and for *F. oxysporum* (*3*).

In general, the nitrogen in the mycelium tends to decrease slightly with age, indicating that old cultures contain less active protoplasm than fresh cultures. These results agree with those obtained for *Fusarium lini* (*1*).

Carbohydrates contain about 40 percent of carbon, proteins 51 to 55 percent, and a typical fat such as tristearin 76.87 percent. During the first 43 days of growth the carbon content of the mycelium ranged from 45.62 to 48.56 percent. During the final stages of growth the carbon content of the mycelium dropped to between 40.00 and 42.67 percent. Since the percentage of nitrogen in the mycelium decreased together with the percentage of carbon it appears that the carbohydrate content of the mycelium was increasing with age. There was no evidence of fat formation, as was the case with *Fusarium lini* (*1*) and *F. oxysporum* (*3*).

RATIO OF PRODUCTS OF METABOLISM TO ONE ANOTHER AND TO THE GLUCOSE CONSUMED

Various coefficients and equivalents have been introduced into the literature to express the relationships existing among the products of metabolism and the compounds consumed by a fungus. A number of these ratios have been calculated for each series of experiments in the present investigation; the ones for the last series are presented in table 2. To avoid confusion, the exact meaning of each ratio is given at the top of each column.

The respiration coefficient represents the grams of carbon dioxide produced per gram of dry mycelium. There is an increase in this ratio with age up to a certain point, and then a slight decrease, this point usually coinciding with the exhaustion of the glucose and the beginning of the utilization of ethyl alcohol. Evidently *Fusarium lycopersici* is able to use ethyl alcohol better for mycelium building than as a source of energy. *F. lini* (*1*) and *F. oxysporum* (*3*) showed a steady increase in this ratio.

TABLE 2.—*Quantitative relationships existing between the various metabolic products of Fusarium lycopersici when grown on a glucose medium*

Age of culture (days)	Respiration coefficient: $\frac{\text{Weight of CO}_2}{\text{Weight of mycelium}}$	Economic coefficient: $\frac{\text{Weight of sugar consumed}}{\text{weight of mycelium}}$	Respiration equivalent: $\frac{\text{Carbon of CO}_2\times 100}{\text{carbon consumed}}$	Plastic equivalent: $\frac{\text{Carbon of mycelium}\times 100}{\text{carbon of glucose consumed}}$	Alcohol equivalent: $\frac{\text{Carbon of alcohol}\times 100}{\text{carbon of glucose consumed}}$	$\frac{\text{Carbon of alcohol}}{\text{Carbon of CO}_2}$
8	2.08	6.83	20.60	24.72	22.70	1.10
15	3.39	12.46	18.50	9.57	32.49	1.76
22	4.07	14.42	19.25	7.92	40.20	2.09
29	4.72	16.02	20.10	7.59	40.70	2.00
37	4.83	15.23	21.56	7.59	35.40	1.64
43	5.22	15.19	23.46	7.54	35.95	1.53
49	5.88	17.15	23.37	6.11	30.15	1.29
56	5.76	16.00	24.52	6.22	27.48	1.12
60	5.77	16.04	24.60	6.66	26.17	1.07

Similar changes take place in the economic coefficient, which is the amount of carbon source required to make 1 g of dry mycelium, the ratio increasing to a maximum and then decreasing as the fungus changes from glucose to alcohol as a building material. *Fusarium lini* (*1*) showed similar changes, but the maximum value was only 13.1, as compared with 17.15 in the case of *F. lycopersici*. Evidently the former organism uses carbon sources more efficiently than the latter. That young mycelium is more efficient in sugar consumption than older mycelium is illustrated by the low value obtained in every case for the first growth period, usually a week or 10 days.

As would be expected, the percentage of the carbon consumed that is transformed into carbon dioxide, or the respiration equivalent, increases with age during the growth of the fungus, since ethyl alcohol is used as a source of energy after the glucose has disappeared.

The plastic equivalent is really the reciprocal of the economic coefficient, since it represents the percentage of the glucose consumed that is converted into mycelium. As would be expected, it shows reciprocal changes, decreasing with age until the glucose is entirely used, and then increasing slightly. These changes also tend to show that ethyl alcohol is used somewhat more efficiently than glucose for mycelium production.

The alcohol equivalent is the percentage of glucose consumed that is converted into ethyl alcohol. The changes in this ratio show that *Fusarium lycopersici* utilizes ethyl alcohol after it has used all the glucose, since the values increase until the point at which the glucose is exhausted is reached, after which they decrease markedly.

In the last column of table 2 the ratio of the carbon in alcohol to the carbon in carbon dioxide is given. In a typical alcoholic fermentation the ratio of carbon in alcohol to carbon in CO_2 is 2:1. Table 2 shows that the experimentally determined ratio approaches very closely the theoretical, and is exactly 2.00 on the day of maximum alcohol production. Because of the utilization of the alcohol by the fungus, the ratio decreases to 1.07:1 on the sixtieth day of growth, indicating that about 25 percent of the alcohol is changed into carbon dioxide. The actual decrease in alcohol is over 30 percent, the difference being accounted for by the growth of the mycelium.

UTILIZATION OF ETHYL ALCOHOL

Since *Fusarium lycopersici* utilizes the ethyl alcohol which it produces from glucose, the effect on the growth of the fungus of various concentrations of ethyl alcohol, both alone and in the presence of the same concentration of glucose that was used in the preceding work, was studied. Two series of 26 flasks each were used, each flask in one series containing 100 ml of mineral nutrient medium without glucose, and each flask in the other series containing the same amount of medium with enough glucose added to make an approximately 2-percent glucose solution. The exact amount of glucose was not determined, but was assumed to be the same in all flasks containing it, since the medium for each flask was measured from one large lot. To 24 flasks from each series, amounts of 95 percent ethyl alcohol varying from 0.2 to 6.0 ml were added after sterilization. Two flasks were used as controls, no alcohol being added. After 10 days growth, the mycelium was removed by filtration and dried to constant weight at 100° C. The results are presented in figure 4.

In low concentrations, ethyl alcohol is a good source of carbon for *Fusarium lycopersici*. The fungus will grow slightly, even in concentrations as high as 4.90 g per 100 ml of solution. That maximum growth occurred at a concentration of 0.41 g per 100 ml is significant, as the maximum amount of alcohol produced by the fungus in any of the studies of the glucose metabolism was 0.57 g per 100 ml. The limiting value for maximum growth is apparently between these two figures. These results compare favorably with those of Anderson (*1*), who obtained maximum growth of *F. lini* at an alcohol concentration of 0.38 g per 100 ml. A concentration of 3.59 g per 100 ml, however, inhibited completely the growth of this organism.

As was expected, growth was generally heavier in the flasks containing glucose in addition to alcohol, although the two growth curves tend to parallel each other. That low concentrations of alcohol have a stimulating effect on the growth of the fungus on glucose is evidenced by the fact that the weight of mycelium produced on the solution containing 0.16 g of alcohol is significantly greater than that on the glucose solution alone. As the amount of alcohol increases, however, the weight of mycelium decreases until, at a concentration of 4.90 g per 100 ml, less mycelium is produced than on a solution containing 4.08 g of alcohol and no glucose.

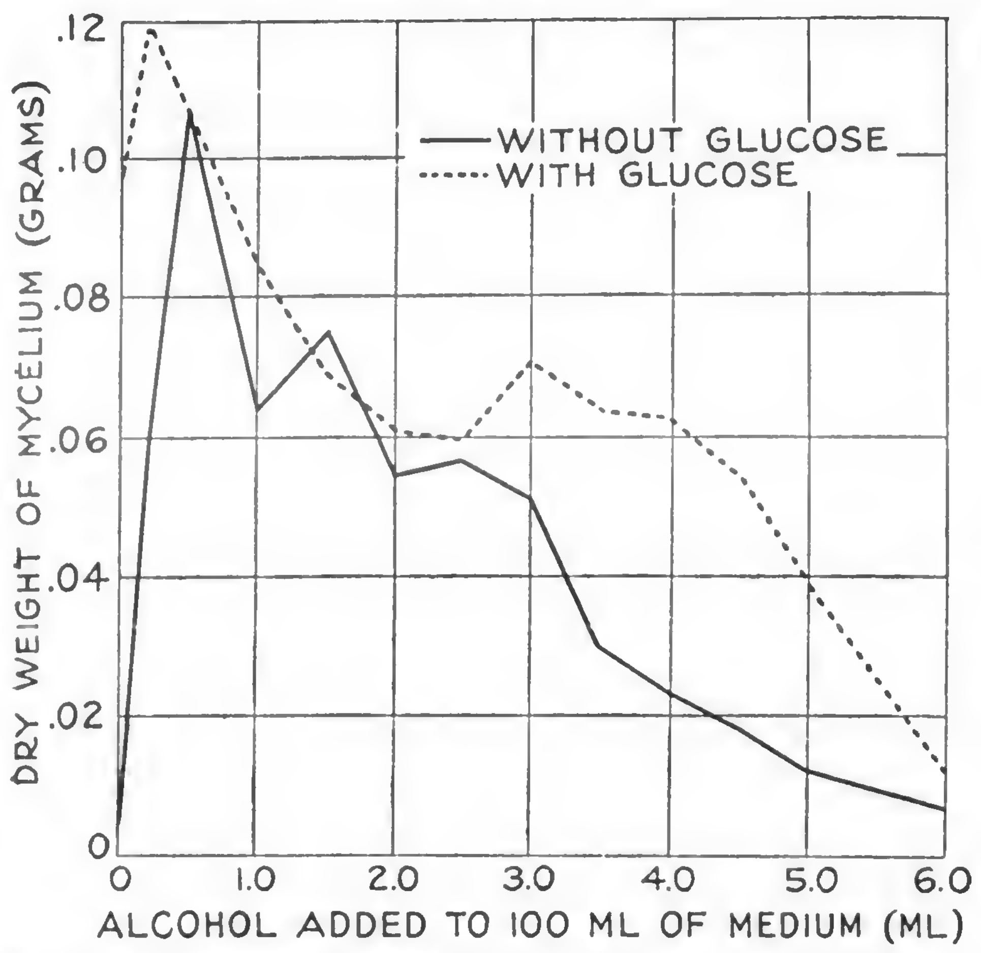

FIGURE 4.—Mycelium production on various concentrations of ethyl alcohol, with and without glucose.

DISCUSSION OF RESULTS

Fusarium lycopersici is as tolerant to extremes of hydrogen- and hydroxyl-ion concentration as *F. lini*, and the optimum pH value for growth is practically the same for both organisms. *F. lycopersici* is apparently better able to change the pH of strongly acidic solutions toward the optimum value than is *F. lini*. In view of the ability of the organism to grow over such a wide range of pH values, it would not appear feasible to attempt to control the *Fusarium* wilt of tomatoes by adjusting the pH of the soil, although Sherwood (*14*) found that there was a greater percentage of infection of tomato seedlings by the fungus in acid soils.

The results of the work on the carbon metabolism of *Fusarium lycopersici* on glucose indicate that this organism produces, in addition to ethyl alcohol and carbon dioxide, appreciable quantities of volatile and nonvolatile compounds, part of which was determined by new methods. Because of the striking esterlike odor of old cultures, it is thought that fatty acids comprise an appreciable portion of these compounds, although attempts to identify these acids were unsuccessful.

The production of these compounds is an essential point of distinction between this organism and *Fusarium lini* and *F. oxysporum*. In the work with these last two fungi over 90 percent of the original carbon could be accounted for in the ethyl alcohol, carbon dioxide, and mycelium, whereas in the present study the maximum percentage of the original glucose that could be recovered in the form of these three products was less than 70 percent. This difference in the percentage recovery probably is due to actual differences in the biological characteristics of the organisms and not to faulty technique.

Another biochemical difference between the three species of *Fusaria* under discussion appears in the carbon content of old mycelia. In *Fusarium lycopersici* the carbon content of the mycelium approaches that of a carbohydrate, whereas with *F. lini* and *F. oxysporum* there is an indication of fat deposition with age.

The course of the alcoholic fermentation caused by *Fusarium lycopersici* is practically the same as that of *F. lini* and of *F. oxysporum*, alcohol and carbon dioxide being produced in approximately the same proportions by all three fungi. The amount of alcohol produced by *F. lycopersici*, however, was somewhat less than that produced by the other two organisms. The maximum quantity obtained in this work was 1.7 g, as compared with 2.1 to 2.2 g in the other investigations.

No attempts were made to discover the substances that are toxic to tomato plants. It does not seem likely, however, that the organism produces alcohol in sufficient quantity to account for its wilting effect, as the maximum concentration obtained in these studies was 0.57 g per 100 ml, and Luz (*7*) found that tomato plants were not wilted when placed in alcohol solutions containing 2 g per 100 ml. White (*17*) found that tomato cuttings were not wilted by concentrations of alcohol up to 4 percent by volume, but that the low molecular weight fatty acids and oxalic acid were highly toxic in concentrations of only 0.06 percent. Concentrations of alcohol of 4 percent or more inhibited the growth of the fungus, even in the presence of glucose, which is presumably an easily assimilable form of carbon; hence it is not likely that the concentration of alcohol in the tissues of the infected plant would become great enough to cause wilting and death of the plant.

SUMMARY

Fusarium lycopersici, the organism which causes tomato wilt, is not sensitive to extremes of hydrogen-ion and hydroxyl-ion concentration. It will grow on artificial media at pH values between 1.88 and 11.06.

The range for good growth is also wide, extending from pH 2.0 to pH 9.5.

The organism shows a decided tendency to change the pH of both acid and alkaline media toward the optimum value, the final pH in five cases varying only between pH 4.25 and pH 4.95.

The principal products of the metabolism of the organism on glucose are carbon dioxide and ethyl alcohol, but considerable quantities of other compounds, presumably volatile and nonvolatile organic acids, are formed as well. Some of the carbon in these unknown compounds was determined by means of new procedures.

The ratio of the carbon in alcohol to carbon in carbon dioxide is very nearly that required by the equation for a typical yeast fermentation. Because of the consumption of the alcohol by the fungus during the later periods of growth, however, this ratio gradually decreases with the age of the culture.

Fusarium lycopersici definitely uses the ethyl alcohol that it produces for energy and growth as soon as the glucose in the solution has been exhausted. That it uses the alcohol somewhat more efficiently than the glucose is evidenced by the slow decrease in the plastic equivalent while the organism is using glucose and the sudden increase in this ratio when alcohol becomes the carbon source.

Ethyl alcohol will serve as the sole carbon source for *Fusarium lycopersici*, maximum growth being obtained on a concentration of alcohol approximating the maximum amount produced by the fungus on a glucose medium. Concentrations of alcohol of 4 percent by volume or more seriously inhibited the growth of the fungus. A small amount of alcohol (0.2 percent) stimulated mycelium production on a glucose medium, whereas concentrations of 5 percent by volume or more inhibited growth.

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For the purpose of this study, dye solut
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Standardization of 2,6-Dichlorophenolindophenol

An Improved Method

M. H. MENAKER AND N. B. GUERRANT
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SEVERAL methods have appeared in the literature for the standardization of 2,6-dichlorophenolindophenol solution as used in the chemical method for the determination of ascorbic acid. Basically these methods all require the standardization of lemon juice or a solution of ascorbic acid against a standard iodine solution, followed by the standardization of the dye against either the lemon juice or the ascorbic acid solution.

There are several objections to the use of such a method. Among these are the instability of the iodine solution, the necessity of preparing fresh lemon juice or fresh ascorbic acid solution every day, the problem of interfering substances when lemon juice is used, and the difficulty at times encountered in obtaining a satisfactory end point.

The method presented here is designed to minimize these disadvantages.

For the purpose of this study, dye solutions were prepared as follows:

The desired quantity of dye (35 to 70 mg. per 100 ml. of solution) is placed in a small beaker and successive portions of hot water are added. After each addition of water the solution is decanted through a filter into a volumetric flask, and when all the dye has been dissolved the filter is washed with small portions of hot water until the washings are colorless or nearly so. After cooling to room temperature, the solution is made up to volume.

Fifteen milliliters of the dye solution are pipetted into a 50-ml. Erlenmeyer flask, 0.5 to 1.0 gram of potassium iodide and 0.5 to 1.0 ml. of dilute sulfuric acid (1 to 4) are added, and, after shaking to facilitate the oxidation of the potassium iodide, the liberated iodine is titrated with 0.01 *N* sodium thiosulfate using the usual starch indicator.

It has been established that 1 ml. of 0.01 *N* iodine solution is equivalent to 0.88 mg. of ascorbic acid; consequently, 1 ml. of 0.01 *N* sodium thiosulfate solution should also be equivalent to 0.88 mg. of ascorbic acid. This has been found to be the case.

Figure 1 represents the data obtained by titrating different concentrations of the dye solution against lemon juice, ascorbic acid, and sodium thiosulfate.

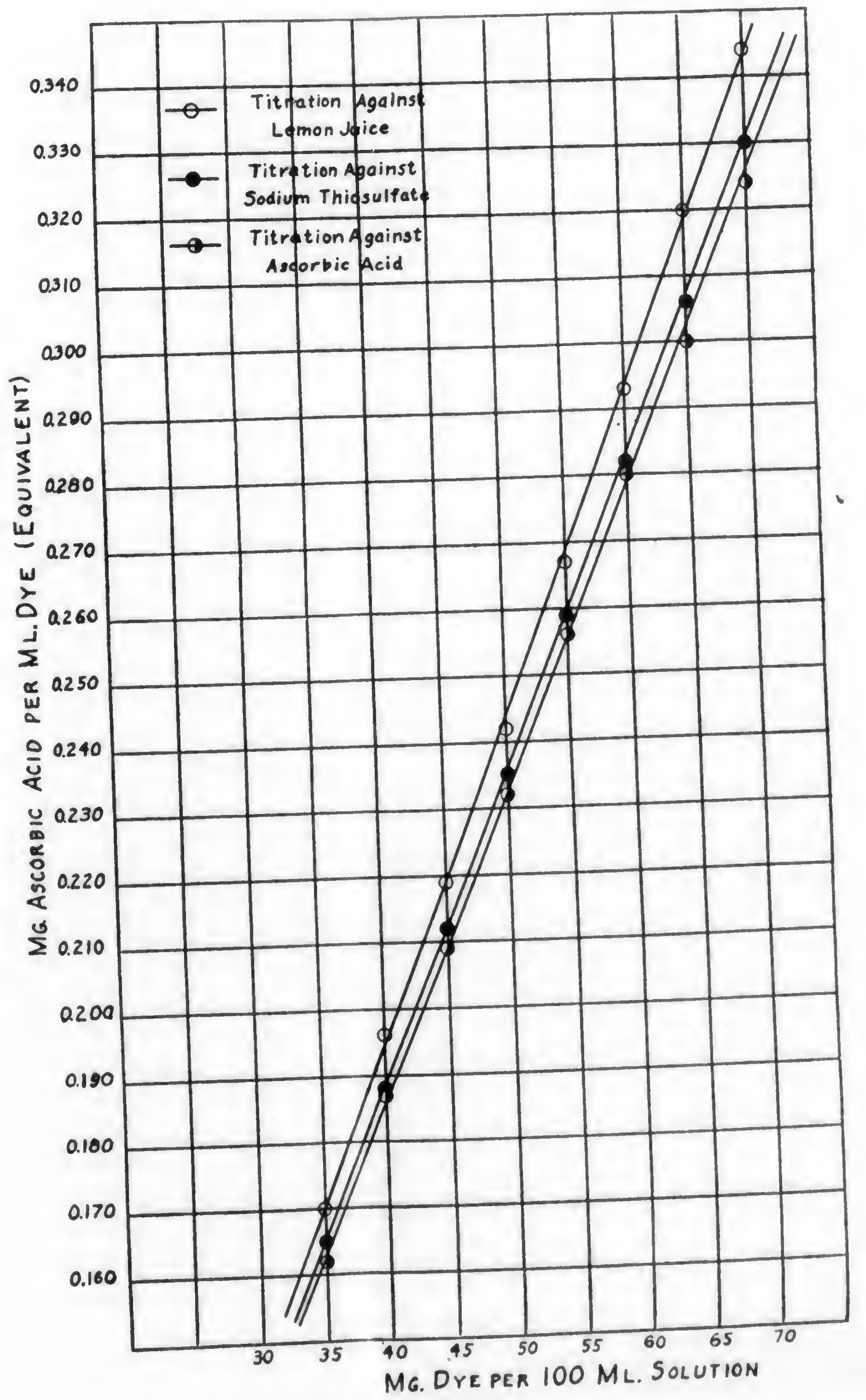

FIGURE 1

Discussion

The curve obtained for the titrations against sodium thiosulfate is comparable with that in which ascorbic acid was used, but is somewhat lower than that obtained with lemon juice. This leads to the conclusion that lemon juice contains small amounts of substances other than ascorbic acid which are oxidized by iodine but not by the dye.

The following equations were obtained by application of the method of least squares to determine the best straight line for each series of points:

For titration against lemon juice,
$$y = 0.004964x - 0.005$$

For titration against ascorbic acid,
$$y = 0.004610x + 0.002$$

For titration against sodium thiosulfate,
$$y = 0.004711x$$

It is interesting to note that the *y* intercept of the curve representing titrations against sodium thiosulfate is zero.

The chief advantages of the proposed method are: The sodium thiosulfate solution remains stable after it has once reached equilibrium; the end point of the titration is sharp as contrasted to the blue-to-pink-to-colorless end-point change observed in the previous methods; and since standard sodium thiosulfate solution is normally required as a check on the standard iodine solution used in either of the other methods, the need for one of the usual standard solutions (the iodine solution) has been eliminated.

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Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 35, No. 1, January, 1938

A23. *A Bacteriological Study of the Bulk Fermentation of Cigar Leaf Tobacco.*

D. W. McKINSTRY, D. E. HALEY AND J. J. REID, Pennsylvania Agricultural Experiment Station, State College, Pa.

A study has been made of the bulk fermentation of cigar leaf tobacco in the warehouses of a manufacturer. Plate counts of fungi and bacteria were made daily and isolations of predominant types were classified and studied. The fermentation of more than one hundred tons of tobacco, representing the products of all the important cigar leaf districts in the United States, was included in this investigation.

Plate counts of tobacco, made previous to the fermentation, revealed from 2000 to 10,000 fungi per gram. Viable fungi were rarely found following the bulking of the tobacco. Bacterial plate counts, made previous to the fermentation, ranged from values of 50,000 to 250,000. Following the water treatment of the tobacco the counts ranged from 3,000,000 to 5,000,000 per gram. The plate counts exceeded 1,000,000,000 shortly after the bulking of the tobacco. Direct counts increased from 500,000,000, previous to the water treatment, to counts in excess of 10,000,000,000 per gram during the final stages of the fermentation.

Variants of *Bacillus subtilis* were found to be the predominant organisms in a satisfactory fermentation of the leaf. Other members of this genus and cocci were found in lesser numbers.

Reprint from Poultry Science: Vol. XVII, No. 1
January, 1938

Fishy Flavor and Odor in Turkey Meat[*]

D. R. Marble, J. E. Hunter, H. C. Knandel and R. A. Dutcher
The Pennsylvania State College, State College, Pennsylvania

(Presented at Annual Meeting, August 1937; received for publication August 12, 1937)

THE use of fish liver oils and fish meals has presented a problem to poultry producers on account of fishy flavor imparted to the meat. They are important ingredients in turkey mashes because the fish liver oil is a source of vitamins A and D and fish meal is an excellent source of protein.

In an attempt to obtain the benefits accruing from the use of fish oils and fish meals and at the same time avoid undesirable flavors it has been the practice of many growers to discontinue the use of fish meal and fish oil in the ration for sometime prior to marketing. This practice necessitates a greater number of mash mixtures. The elimination of the vitamin D oil also may cause rachitic symptoms in the growing turkeys when they are kept in confinement.

Carrick and Hauge (1926) have reported the occurrence of fishy flavors in chicken meat. A press release of the United States Department of Agriculture in December, 1936, reported fishy flavors in turkey and chicken meat. Hauge and Carrick studied the effect of cod liver oil and workers in the United States Department of Agriculture studied the effect of cod liver oil and fish meal singly and in combination. In the study herein reported the objective was to determine (1) the effect upon flavor and odor of cod liver oil (not U.S.P.) and white fish meal and (2) the proper time to discontinue feeding these ingredients in advance of slaughtering in order to prevent the occurrence of fishy flavor and odor in the meat.

EXPERIMENTAL PROCEDURE AND DATA

Bronze turkeys were used in this study and they were fed on the following turkey starter for eight weeks.

28 pounds	yellow corn meal
10 pounds	wheat bran
10 pounds	wheat middlings
10 pounds	ground heavy oats
5 pounds	alfalfa meal
10 pounds	dried skimmilk
15 pounds	meat scrap
10 pounds	fish meal
1 pound	salt
1 pound	cod liver oil
100 pounds	total

At eight weeks of age the poults were divided into four groups of 12 poults each. Each group received an all mash ration containing approximately 20 percent protein. Group 1 received a basal ration containing no cod liver oil or fish meal. Group 2 was fed a similar ration except that 1 per cent of a poultry grade cod liver oil assaying 100 U.S.P. units per gram was added to the mash. Group 3 received the basal ration without cod liver oil but with 10 percent white fish meal added in place of meat scraps. Group 4 was given a ration containing 1 percent cod liver oil and 10 per cent fish meal. The fish meal used was a vacuum dried white fish meal. Table 1 shows the composition of the various rations used.

In order to determine the length of time before slaughtering necessary to remove the fish meal or cod liver oil from the ration three birds from Groups 2, 3 and 4 were

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[*] Authorized for publication July 27, 1937 as paper No. 779 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

TABLE 1.—*Composition of experimental rations*

	Group 1 Basal	Group 2 Basal + oil	Group 3 Basal + fish meal	Group 4 Basal + oil and fish meal
Yellow Corn Meal	31	30	31	30
Wheat Bran	15	15	15	15
Wheat Middlings	15	15	15	15
Ground Heavy Oats	10	10	10	10
Alfalfa Meal	5	5	5	5
Dried Skim Milk	5	5	5	5
Meat Scrap	17	17	7	7
White Fish Meal	—	—	10	10
Salt	1	1	1	1
Ground Limestone	1	1	1	1
Cod Liver Oil (Poultry grade not U.S.P.)	—	1	—	1
	100	100	100	100

taken from their respective pens and placed in the basal group, at 20, 24 and 26 weeks of age. This procedure gave thirteen feeding combinations. When the birds were 28 weeks of age, one male and one female or a total of twenty-six birds were slaughtered and cooked. Each bird was cooked at the same temperature, without seasoning, in an individual oven. The females were cooked on one day and the males were cooked one week later.

Nineteen individual tasters coöperated in the scoring of the birds for fishy flavor and odor. The treatment of the birds was not disclosed to the tasters and the tasters were asked not to confide their observations with others until after the score sheets were turned in. Both men and women participated in the scoring. The tasters scored each bird for flavor and odor and listed their reactions as none, slight, medium or strong. They observed odor of the carcass as a whole and scored the breast meat, thigh meat and drippings for flavor. Cutlery was washed with hot water after each tasting and the tasters ate a small piece of dry bread and drank a small amount of black coffee in order to remove the taste from their mouths before proceeding with the next sample.

TABLE 2.—*Distribution of scores made by 19 observers for fishy flavor in the breast meat of carcasses of 28-week old turkeys*

Substitutions in Basal Ration	Period Experimental Ration Was Fed	Period Basal Ration Was Fed	Females: Degree of Fishy Flavor: None	Slight	Medium	Strong	Males: Degree of Fishy Flavor: None	Slight	Medium	Strong
	Weeks	Weeks	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores
None	—	9–28	18	1			19			
1% Cod Liver Oil	9–20	21–28	19				19			
	9–24	25–28	18	1			18	1		
	9–26	27–28	15	3	1		19			
	9–28	—	11	5	2	1	13	6		
10% White Fish Meal	9–20	21–28	19				19			
	9–24	25–28	18	1			19			
	9–26	27–28	14	5			19			
	9–28	—	16	3			19			
1% Cod Liver Oil Plus 10% White Fish Meal	9–20	21–28	18	1			19			
	9–24	25–28	17	2			19			
	9–26	27–28	4	9	5	1	10	8	1	
	9–28	—	12	3	3	1	10	6	3	

It was originally planned to score the carcasses immediately after removal from the oven and then again a few hours later when the carcasses had become cold. This was done with the female carcasses but because a close correlation existed between the scorings of the hot and cold meat, the male carcasses were scored only when hot.

TABLE 3.—*Distribution of scores made by 19 observers for fishy flavor in the thigh meat of carcasses of 28-week old turkeys*

Substitutions in Basal Ration	Period Experimental Ration Was Fed	Period Basal Ration Was Fed	Females: Degree of Fishy Flavor: None	Slight	Medium	Strong	Males: Degree of Fishy Flavor: None	Slight	Medium	Strong
	Weeks	Weeks	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores
None	—	9–28	17	2			19			
1% Cod Liver Oil	9–20	21–28	15	4			19			
	9–24	25–28	13	4	1	1	17	2		
	9–26	27–28	11	5	3		17	2		
	9–28	—	6	6	5	2	5	7	3	4
10% White Fish Meal	9–20	21–28	17	2			19			
	9–24	25–28	16	3			17	2		
	9–26	27–28	7	10	2		16	3		
	9–28	—	13	4	2		14	5		
1% Cod Liver Oil Plus 10% White Fish Meal	9–20	21–28	13	4	1	1	16	3		
	9–24	25–28	9	5	1	4	8	10	1	
	9–26	27–28	—	6	5	8	3	6	8	2
	9–28	—	2	7	3	7	1	4	8	6

Table 2 presents the distribution of scores made by the 19 observers on the breast meat. A relatively small number of tasters detected a fishy flavor in the breast meat of carcasses of the turkeys fed 1 percent cod liver oil or 10 percent of white fish meal when they were removed from the ration four weeks prior to slaughtering. When, however, either cod liver oil or white fish meal was left in the ration until slaughtering or two weeks prior to slaughtering some observers noted a fishy flavor in the breast meat. This appeared to be more serious in the birds fed cod liver oil than in those receiving the white fish meal. A more pronounced taste appeared when white fish meal and cod liver oil were fed in combination.

Table 3 presents the scores made for fishy flavor in the thigh meat. A greater degree of fishy flavor was observed in the thigh meat than in the breast meat. This is apparent in all three groups which received a substitution in their mash mixture. However, the tendency for one percent of a poultry grade of cod liver oil to give a more pronounced fishy flavor than a 10 percent level of white fish meal is observed also in the scoring of the thigh meat.

In addition to tasting a portion of the breast and thigh meat, the drippings were tasted on a glass rod dipped into the fat rendered from the birds in cooking. The distribution of the scores as presented in Table

TABLE 4.—*Distribution of scores made by 19 observers for fishy flavor in the drippings of carcasses of 28-week-old turkeys*

Substitutions in Basal Ration	Period Experimental Ration Was Fed	Period Basal Ration Was Fed	Females				Males			
			Degree of Fishy Flavor				Degree of Fishy Flavor			
			None	Slight	Medium	Strong	None	Slight	Medium	Strong
	Weeks	Weeks	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores
None	—	9–28	16	2	1		17	2		
1% Cod Liver Oil	9–20	21–28	14	3	1	1	17	2		
	9–24	25–28	12	5	2		15	4		
	9–26	27–28	10	4	5		19			
	9–28	—	2	8	4	5	2	4	8	5
10% White Fish Meal	9–20	21–28	16	3			17	2		
	9–24	25–28	13	5	1		14	5		
	9–26	27–28	3	9	6	1	12	5	2	
	9–28	—	11	5	2	1	11	3	4	1
1% Cod Liver Oil Plus 10% White Fish Meal	9–20	21–28	12	5	2		13	6		
	9–24	25–28	7	4	7	1	6	6	5	2
	9–26	27–28		1	4	14		3	6	10
	9–28	—		5	4	10		1	5	13

TABLE 5.—*Distribution of scores made by 19 observers for fishy odor in the carcasses of 28-week-old turkeys*

Substitutions in Basal Ration	Period Experimental Ration Was Fed	Period Basal Ration Was Fed	Females				Males			
			Degree of Fishy Odor				Degree of Fishy Odor			
			None	Slight	Medium	Strong	None	Slight	Medium	Strong
	Weeks	Weeks	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores	No. Scores
None	—	9–28	14	5			19			
1% Cod Liver Oil	9–20	21–28	14	4	1		17	2		
	9–24	25–28	14	5			15	4		
	9–26	27–28	11	3	5		17	1		1
	9–28	—	5	5	5	4	4	7	6	2
10% White Fish Meal	9–20	21–28	16	3			18	1		
	9–24	25–28	16	3			15	4		
	9–26	27–28	7	10	1	1	12	7		
	9–28	—	13	4	2		12	5	1	1
1% Cod Liver Oil Plus 10% White Fish Meal	9–20	21–28	14	5			16	3		
	9–24	25–28	9	5	4	1	11	4	3	1
	9–26	27–28	1	6	4	8	3	5	7	4
	9–28	—	3	6	5	5	2	3	5	9

4 showed an even more pronounced fishy flavor in the drippings than was recorded for the breast or thigh meat. The fishy flavor became very pronounced in the drippings from carcasses of the turkeys fed both cod liver oil and white fish meal. The fishy flavor frequently could be detected in the drippings even though the meat was devoid of fishy taste.

When both white fish meal and cod liver oil were eliminated from the ration eight weeks prior to slaughtering, the fishy flavor in the drippings was not pronounced although several tasters detected it. When these materials were eliminated from the ration two weeks prior to slaughtering or fed until slaughtering time all tasters recorded some degree of fishy flavor in the drippings.

Table 5 presents the results of scoring the carcasses for fishy odor. The data for odor closely correlated those for flavor.

DISCUSSION

Since other taste studies have shown a wide variation in the taste reaction of individuals an effort was made to use as many tasters as feasible. The studies herein reported also show a wide variation in the taste reaction of individuals. This was true with both men and women tasters. Perhaps some individuals imagined that they detected the flavor of fish or failed to properly remove the previous taste by use of coffee and bread. One taster reported the flavor of fish in some part of every female carcass. However, she reported a more pronounced flavor in the carcasses identified by others as carrying the fishy flavor.

More pronounced flavor and odor were apparent in the female turkey carcasses than in the males. This probably can be explained by the fact that when the turkeys were removed from the groups receiving fish meal and cod liver oil and placed on the basal ration, the females were in much better flesh than the males, and consequently carried more of the fat soluble factor or factors responsible for fishy flavor and odor.

SUMMARY

Twenty-six carcasses of turkeys, which were fed cod liver oil, fish meal or both for varying periods of time, were roasted in separate ovens. The degree of fishy flavor in the breast meat, thigh meat and drippings and the odor of the carcass as a whole was scored by nineteen observers.

Fishy flavor and odor were observed in the roasted carcasses of turkeys 28 weeks of age fed either 1 percent of a poultry grade cod liver (not U.S.P.), 10 percent of vacuum dried white fish meal or both during the entire growth period. The combination of these two ingredients in the mash produced a more pronounced flavor and odor than when fed separately.

Three birds were removed from the groups receiving either cod liver oil, fish meal or the combination of both at 8, 4, and 2 weeks prior to slaughter and placed in the group receiving the basal ration containing neither cod liver oil nor white fish meal. The removal of both cod liver oil and white fish meal from the diet eight weeks prior to slaughtering time practically eliminated the occurrence of both fishy flavor and odor.

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capacity, about 15,400 lbs (7,700 qts., 1,925 gals.). I suppose that one would have been recommended to churn 2,500 or 3,000 liters of cream, say 700 of our gallons of cream.

The cream ripeners exhibited and those used in Europe are almost entirely of the swinging coil type with the bearing above the cream.

There were exhibits of centrifugal cream separators, much like those at our machinery exhibition at Atlantic City, comprising—

Alfa Separators from Sweden,
Titan Separators from Denmark, and
Westfalia Separators made in Germany.

I was impressed by the pains taken in Sweden and Denmark to see that the butter that is exported from those countries shall meet very high standards. Butter that is not made of proper materials, or that is below standard in any way, cannot bear the State Brands required for export butter. I was also surprised to learn of a new law recently passed in Denmark, requiring oleomargarine to contain a certain amount of vitmin "A."

I greatly appreciated the opportunity of attending the World's Dairy Congress and meeting and talking with others from different countries and learning of their experiences.

RECENT TRENDS IN VITAMIN RESEARCH

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VITAMIN A

Practically every one connected with the dairy industry is familiar with the relatively recent discovery that the orange yellow pigment known as carotene is the parent substance or precursor of vitamin A. It is now known that it is not necessary for animals and human beings to ingest foods containing vitamin A if the supply of this pigment (carotene) is present in the diet in sufficient quantities. It is possible for the tissues of the body, particularly liver tissues, to change carotene into vitamin A for the physiological needs of the body.

This discovery has stimulated many interesting and practical types of research which I desire to mention briefly. After chemists found that it was possible to prepare pure crystalline carotene in amounts sufficient for experimental work, it was discovered that this pigment possessed properties characteristic of vitamin A when fed to experimental animals. In the meantime, the organic chemist had been able to determine the exact chemical structure of carotene. Just as soon as this information became available, the organic chemist and the biological chemist postulated that vitamin A must be chemically related in some way to carotene. It was not long before the chemist postulated the probable structure of vitamin A and predicted that one molecule of carotene, $C_{40}H_{56}$, could be broken down into two molecules of vitamin A with the formula $C_{20}H_{29}OH$. It has been found that there are several carotene pigments, one of which, known as beta carotene, is the most potent since it yields the largest amount of vitamin A per unit of weight. Within the past two years the organic chemist has been able to synthesize vitamin A in the chemical laboratory, proving the correctness of the above mentioned prediction. It is now possible to write the chemical formula for vitamin A with the same confidence that we have been writing formulae of many commonly known substances.

It has been known for many years that the principal yellow pigment in cow's milk is the fat soluble carotene which is obtained from the plant materials eaten by the cow. Since carotene is the parent substance of vitamin A, it is but natural to postulate that the highly pigmented milks from Guernsey and Jersey cows should be more valuable from the standpoint of vitamin A content than the milks of the less pigmented breeds such as Holsteins and Ayrshires. Our coöperative research work with Dr. S. I. Bechdel at the Pennsylvania State College has shown that the less pigmented breeds ingest just as much or more carotene in their feed but deposit less of this pigment in their tissues and excrete less of the pigment in their milk fat. This is due, apparently, to the fact that the less pigmented breeds have greater ability to transform the colored carotene into colorless vitamin A. As a result the total vitamin potency of butter fat, from the various dairy breeds, varies but little. Butter fat from the pigmented breeds was found to be richer in carotene but poorer in colorless vitamin A, while the reverse was true for the less pigmented breeds. Since the latter changed greater amounts of carotene into colorless vitamin A, their milk fat is less pigmented but the nutritional value of the fat has not changed, since the carotene has been transformed to the vitamin A which is needed by the animal body. It would appear, therefore, that the so-called pigmented breeds have a limited capacity for transforming carotene into vitamin A and, as a result, they go through life in what might be called a continuous state of carotinemia. Milk from the pigmented breeds tends to be somewhat richer in vitamin A potency than that of the less pigmented breeds, since the average per cent of butter fat is somewhat higher.

Another problem which has confronted the worker in vitamin research has been to find some satisfactory method of expressing vitamin A potency of food materials. Since it was discovered that beta carotene could be prepared in pure form, it was suggested that this might make a suitable material against

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NOTE.—This is a brief abstract of an informal address delivered at the Annual Banquet on January 18, 1938.

which all food materials might be measured. The sub-committee on public health of the League of Nations sponsored several international conferences with the result that beta carotene has been adopted as the official international standard. Less than one-millionth of a gram (0.6 gamma) of pure beta carotene has been found to bring about certain uniform responses in experimental animals. As a result all laboratories now use reference groups of animals which receive one international unit of vitamin A in the form of beta carotene. Unknown foods are fed to other animals at the same time until it is possible to find that amount of food which will cause experimental animals to respond in a manner similar to that of those animals which receive one unit of the international standard. By this method it is possible for all laboratories over the world to express the vitamin A content of foods in the same terms. These units are known as international units. Since the U. S. Pharmacopoeia is the standard to which all druggists and drug manufacturers must conform, the U. S. Pharmacopoeia experts have adopted the international unit as the "U. S. P. unit." Consequently, these terms are synonymous.

While there is still much to be done by the medical profession in establishing vitamin requirements for human beings, I believe it is safe to state at this time that the vitamin A requirements of human beings will vary from about 3,000 to 6,000 international units (I. U.). Babies usually require from 2,000 I. U. to 5,000 or 6,000 I. U. Some authorities have advocated vitamin A intakes for growing children as high as 9,000 I. U. This is the reason that physicians advocate the use of cod liver oil in child feeding, since good average medicinal cod liver oil will assay 2,000 I. U. per teaspoonful and higher. Butter fat is also variable, depending upon the diet of the cow, and will probably average somewhere between 500 and 1,400 I. U. per ounce. It would appear that mother's milk is usually richer in vitamin A than cow's milk. Most medical authorities are agreed that at least 6,000 I. U. per day are necessary for pregnant and nursing mothers.

A new instrument is being used experimentally in many clinics for the determination of profitable vitamin A deficiency

in humans. This instrument is known as a visual photometer. This instrument measures the speed of recovery of vision after the eye has been subjected to a very intense light. A pigment known as visual purple in the retina of the eye is bleached or destroyed by light. Regeneration of the visual purple appears to depend upon the carotene of vitamin A storage in the tissues. Consequently, a person with ample vitamin A reserves should be able to regenerate visual purple more rapidly than a person who is vitamin A deficient. This has been found to be true within certain limits and it would appear that this instrument will be useful in obtaining semi-quantitative information regarding the vitamin A reserves of the body.

VITAMIN B (B_1)

This vitamin has been isolated in pure crystalline form and only recently has the chemist been able to find methods of synthesizing it artificially in the laboratory. It is possible, therefore, to write the formula for this vitamin. It is known on the market as thiamine or crystalline vitamin B_1 and has the chemical formula $C_{12}H_{17}N_4SC1$. The international unit used in referring to the potency of this vitamin is an arbitrary one based on a standard preparation made from rice polishings. It will not be long before crystalline thiamine will become the international standard. Our vitamin research laboratory at Penn State has been designated as one of the referee laboratories to coöperate with the U. S. Government and the Public Health Committee of the League of Nations with the hope of establishing a definite international standard for vitamin B_1 in the very near future.

It would appear that considerably less than 40 per cent of vitamin B_1 ingested by the cow ever finds its way into the milk. As a result cow's milk cannot be considered a rich source of this vitamin. Using the present tentative international standard, it is possible to state that milk contains from about 70 to 175 international units per quart. The best information obtainable at the present time would indicate that human beings require in the neighborhood of 300 international units of vitamin B_1 per day. There are many individuals who need at least twice that amount.

VITAMIN B$_2$ (G)

This vitamin has been found to be a water soluble pigment found in fairly large concentrations in milk, eggs and liver. It is known as lacto flavin or ribo flavin and has been isolated from these food materials in pure crystalline form and synthesized artificially in the chemical laboratory. While it is not feasible (in this short address) to describe the structure of this vitamin, it is possible to indicate what is known as the empirical formula. The empirical formula for lacto flavin is $C_{17}H_{20}F_4O_6$. This vitamin has attracted considerable attention during the past two years and several industrial concerns are attempting to manufacture it in a commercial way.

To date it has been impossible to establish an international unit for vitamin B$_2$ and the one now in use is known as the Sherman-Bourquin unit. This unit is based on the response of experimental rats under definite controlled conditions. Cow's milk contains from 360 to 720 S-B units per quart and the probable daily requirement for humans is about 400 S-B units per day. The poultry production industry is becoming decidedly flavin conscious and there is a real demand for flavin concentrates at the present time. It is being manufactured from milk, animal tissues, brewery by-products, etc. There is also a need for this particular vitamin in the manufacture of dog foods as well as infant and invalid foods, pharmaceuticals, etc. While the work is barely under way, reports from clinical laboratories and hospitals indicate that there is a definite place for Vitamin B$_2$ in medical practice.

VITAMIN C (ASCORBIC ACID)

Since I last addressed your group this vitamin has been prepared in pure crystalline form and synthesized in the chemical laboratory. It is known as ascorbic acid or cevitamic acid. Ascorbic acid has the formula $C_6H_8O_6$ and can be determined by chemical means.

In 1919 Wisconsin and New York workers corroborated our findings relative to the influence of diet on the vitamin C content of cow's milk. At that time we all contended that the vitamin C content of cow's milk depended upon the amount of vitamin C in the diet of the cow. Workers at the Kansas State College have taken the stand that the vitamin C content of milk cannot be affected by feeding. We feel that diet does affect the vitamin C content of cow's milk within certain limits and work is now being planned at the Pennsylvania State College to repeat this study, using the more refined and accurate chemical technics which have been developed in the last few years.

Pure crystalline ascorbic acid is used as the international standard, 1/20 of a milligram being equal to one international unit. Milk contains from 21 to 36 milligrams of ascorbic acid per quart or from 100 to 180 I. U. It would appear that human beings demand about 300 I. U. per day. This amount cannot be furnished in milk alone and most diets should be supplemented with vegetables, orange juice, tomato juice or similar fruit juices to insure an adequate supply of vitamin C.

VITAMIN D

A few years ago chemists thought that there was but one vitamin D. Within the past two or three years evidence has accumulated which indicates that there are at least nine separate chemical compounds possessing vitamin D activity. So far as the layman is concerned this is not a matter of great importance, since the natural vitamin D and the artificial vitamin D (irradiated ergosterol) are the principal commercial sources at the present time.

The international standard is an arbitrary one based upon the preparation of a pure ergosterol preparation known as calciferol (which has been irradiated with ultraviolet light under controlled conditions). Cod liver oil preparations have been standardized against this international standard and samples of the reference cod liver oil are available for all laboratories which conduct vitamin D assays. It is, therefore, possible for laboratories over the world to speak of the vitamin D content of all food materials in the same terms. It is impossible to go into detail regarding the standardization of the bio-assays other than to state that the U. S. Pharmacopoeia describes standard feeding methods and bone examinations in order that all laboratories may obtain comparable results.

Cow's milk is considered a poor source of vitamin D since it contains from 10 to 40 I. U. per quart. Medical authorities are agreed that babies need not less than 300 I. U. per day and some authorities feel that the daily intake should be as high as 800 for some individuals. The American Medical Association recommends 400 vitamin D units per day for the average child and this is the reason why most vitamin D milks are standardized at 400 vitamin D units per quart. The U. S. Pharmacopoeia requires that a medicinal cod liver oil shall contain not less than 85 I. U. per gram of oil. Most good cod liver oils are in excess of this figure.

Medical authorities are agreed that human beings can use the artificial and natural vitamin D with about equal efficiency. It would appear that there is a slight difference in efficiency of utilization which seems to be slightly in favor of the natural vitamin D. The difference, however, is not sufficiently great to merit emphasis. It would appear, therefore, that the type of vitamin D milk ingested by the child is not a matter of nutritional importance, so long as the child receives an adequate supply of the bone building vitamins.

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THE EFFECT OF THE RIPENING PROCESS ON THE VITAMIN A CONTENT OF CHEDDAR CHEESE*†

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Various types of cheese have long been recognized as valuable adjuvants to the human dietary. Among the several types of cheese used for human consumption, the whole milk cheeses merit special consideration. This is because these cheeses, in addition to being excellent sources of certain minerals and proteins of high biological value, are rich in milk fat. This is especially true of the whole milk cheeses.

Those cheeses which contain relatively large percentages of milk fat might be expected to be rich sources of vitamin A. In fact, a few data have appeared in scientific literature (Cook and Axtmayer (1), Coward and Morgan (2)) which indicate that the Cheddar cheese is an excellent source of this vitamin. There appear to be no data available, however, concerning the relationship of the vitamin A content of the fresh cheese curds to the vitamin A content of the marketable product. Nutritional investigators, working along other lines, have reported that the actions of certain enzymes and bacteria are definitely destructive to carotene and to vitamin A. From such reports it might appear that the vitamin A content of the ripened cheese is only a fraction of the vitamin A content of the products incorporated into the cheese. Since different types of cheese, as well as different batches of the same type, are cured for varying periods of time, depending upon various factors, it would appear that those cheeses which had been subjected to the longer periods of curing and storage should have undergone the greatest depreciation in their vitamin A content. In such cases, the rate and extent of vitamin A deterioration should undoubtedly depend upon a number of factors.

Since several types of cheese are known to contain appreciable quantities of vitamin A, and since no data were available concerning the stability of the vitamin A of cheese during the ripening process, it was believed that a study concerning the changes in the vitamin A content of Cheddar cheese during the ripening process would yield data of value.

EXPERIMENTAL

The investigation herein reported was restricted to a study of the stability of the vitamin A of Cheddar cheese during the ripening process. The cheese

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used in these studies was a portion of a regular batch of Cheddar cheese which had been manufactured in the usual manner by the Department of Dairy Husbandry. This cheese was made from the regular run of raw milk which had been produced during the early days of March. A representative sample of cheese was taken for vitamin A assay at the time the cheese was placed in the ripening chamber and similar samples were taken at intervals during the ripening process. The moisture content of each sample of cheese was determined just previous to the beginning of the assay. During the period of assay, the cheese was maintained in an air-tight container which in turn was kept in an electric refrigerator.

The vitamin A assays were carried out according to the U. S. P. technic (3) with the exception that the assay period was extended to 35 days. Each test animal was maintained at all times in an individual metal cage and was given the usual care and attention indicated by the above technic. The assay groups consisted of from six to twelve carefully selected test animals. Extreme care was exercised in distributing litters, sexes and body-weights uniformly throughout the several assay groups.

Preliminary studies with the original cheese curds at the beginning of the ripening period indicated that daily doses ranging from 0.1 to 0.4 gram were sufficient to insure satisfactory growth and at the same time made sufficient allowance for marked destruction of vitamin A during a prolonged ripening process. Therefore, this range of daily allotments was adopted. Carefully weighed quantities of cheese were fed, in separate containers, to vitamin A deficient animals. While the assays were in progress, a comparable group of animals was being fed a definite unitage of vitamin A in the form of the U. S. P. reference cod-liver oil. It may be stated in connection with the weighing of the daily portions of cheese that these portions were always taken from a freshly cut surface in order to minimize the effect of moisture losses during the assay period.

DATA

Data obtained as the result of this series of assays have been condensed and are presented in the following graphs (Figure 1).

DISCUSSION

From the data presented in the above graphs it appears quite evident that the vitamin A content of Cheddar cheese does not change appreciably during a combined ripening and storage period of one year. A period of this duration is somewhat longer than that commonly used in practical cheesemaking. It appears safe, therefore, to conclude that there is no serious loss in the vitamin A potency of such cheese during the usual curing period.

While the data, as presented above, indicate only a slight decrease in the vitamin A potency of the cheese as a result of the ripening process, this decrease becomes somewhat more significant when the growth responses are adjusted for the difference in moisture content of the various cheese samples

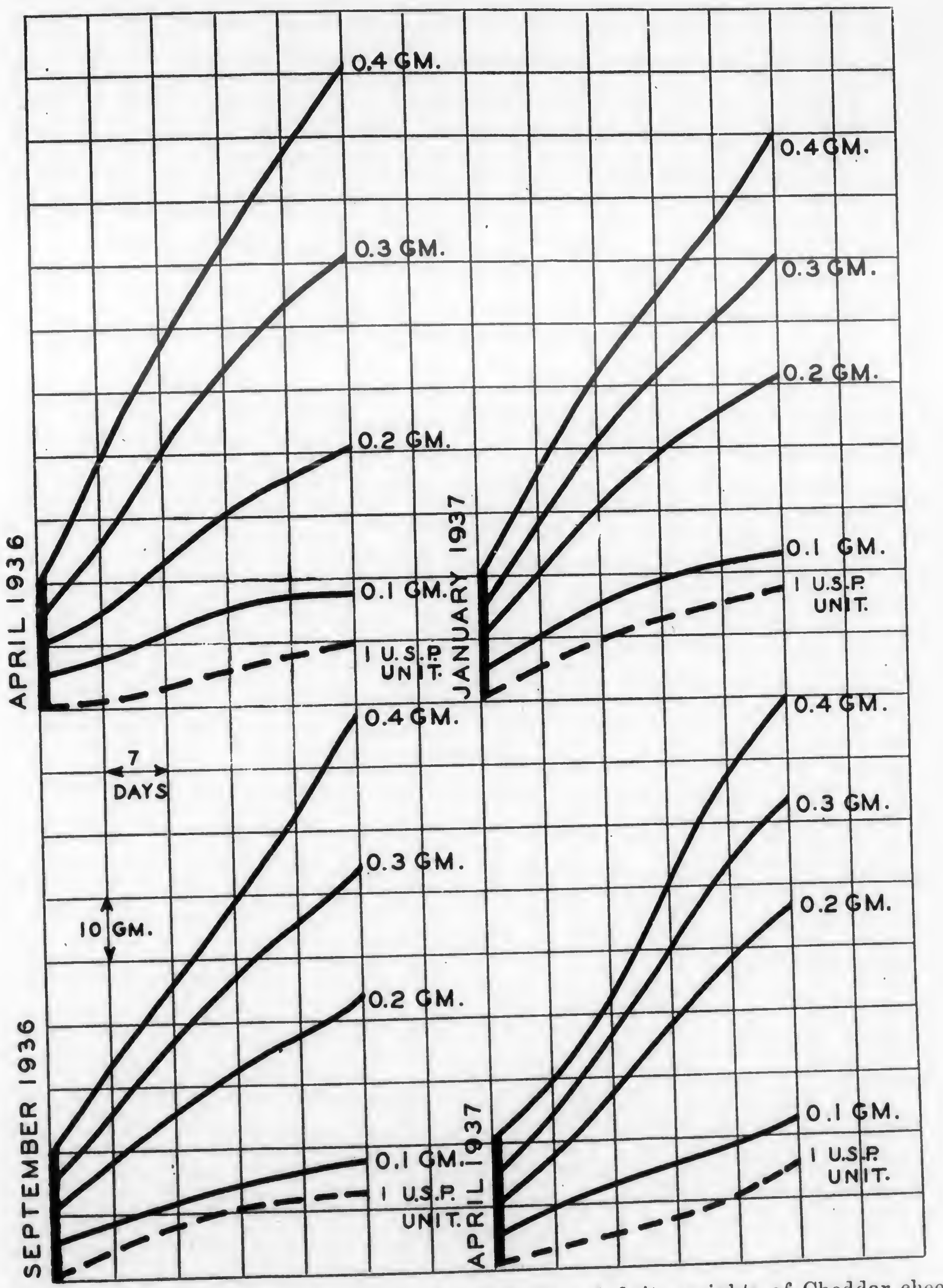

FIG. 1. Growth responses resulting from feeding definite weights of Cheddar cheese, which had been ripened for different periods of time, to groups of vitamin A deficient rats as the only supplement to a vitamin A deficient diet, are given. Corresponding growth responses resulting from feeding a definite unitage of vitamin A in the form of the U. S. P. reference cod-liver oil are also given.

at the time of assay. The moisture content of the four samples of cheese at the beginning of the assay was 37.36, 33.25, 30.29 and 30.00 per cent, respectively. Owing to moisture losses, the amounts of moisture-free cheese fed at the second and subsequent assays were larger than those fed during the initial assay and, therefore, should have produced greater growth responses if there had been no vitamin A destruction. Since the growth responses obtained in the above studies were somewhat less, in spite of the slight increase in the amounts of moisture-free cheese fed, it can only be concluded that there had been a measurable amount of vitamin A destruction during the ripening process. But since these losses in vitamin A potency were so nearly compensated for by the decrease in moisture content of the cheese, the loss in vitamin A units per unit weight of cheese to the purchaser, as a result of the ripening process, appeared to be insignificant.

According to the above data, the finished cheese contained approximately 4500 U. S. P. units of vitamin A per pound. This potency is somewhat less than that reported for Cheddar cheese by other investigators. This difference in potency is believed to be due to differences in vitamin A intake of the cows producing the milk from which the cheese was made. It has long been known that the vitamin A content of milk (milk fat) depends upon the vitamin A intake of the cow producing it. The cheese used in these studies was made from milk which had been produced during the month of March, at a time when the vitamin A intake of the cow is relatively low as compared to other seasons of the year when green pasture, green feeds, etc., are available. The low vitamin A potency of the above cheese is, therefore, attributed to the low vitamin A content of the curds from which the cheese was made. When one considers the fact that cheese is often made from surplus milk and that surplus milk is usually more plentiful during the spring and summer months, it at once becomes apparent that much of the Cheddar cheese, as purchased on the market, may contain a vitamin A potency in excess of that reported above.

SUMMARY

Data are presented to show that no serious destruction of vitamin A occurs during the ripening of Cheddar cheese. While a measurable decrease in vitamin A potency was apparent, this loss was almost wholly compensated for by moisture losses. It appears, therefore, that a pound of Cheddar cheese purchased at the end of the usual ripening period will contain approximately the same amount of vitamin A as a pound of the original curds in spite of the fact that some destruction of the vitamin has occurred.

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- **Bakery Products**
- **Ice Cream**
- **Meat Products**
- **Nut Products**
- **Potato Chips**
- **Other Fatty Foods**

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According to the above data, the finished cheese contained approximately 4500 U. S. P. units of vitamin A per pound. This potency is somewhat less than that reported for Cheddar cheese by other investigators. This difference in potency is believed to be due to differences in vitamin A intake of the cows producing the milk from which the cheese was made. It has long been known that the vitamin A content of milk (milk fat) depends upon the vitamin A intake of the cow producing it. The cheese used in these studies was made from milk which had been produced during the month of March, at a time when the vitamin A intake of the cow is relatively low as compared to other seasons of the year when green pasture, green feeds, etc., are available. The low vitamin A potency of the above cheese is, therefore, attributed to the low vitamin A content of the curds from which the cheese was made. When one considers the fact that cheese is often made from surplus milk and that surplus milk is usually more plentiful during the spring and summer months, it at once becomes apparent that much of the Cheddar cheese, as purchased on the market, may contain a vitamin A potency in excess of that reported above.

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KEEPING QUALITY of Crackers *IMPROVED By Antioxygen*

By HOWARD O. TRIEBOLD *

Department of Agricultural and Biological Chemistry,
The Pennsylvania State College, State College, Pennsylvania

▸Prevention of the development of rancidity, or other changes in which the oxidation of fats or oils plays a part, is of growing importance to food manufacturers. The buying public is becoming steadily more flavor conscious. To obtain and hold a place in the market, the food processor needs to retain the original flavor in his products for as long as possible. Progress in this direction is of interest to manufacturers of

Bakery Products
Ice Cream
Meat Products
Nut Products
Potato Chips
Other Fatty Foods

A SERIOUS PROBLEM is frequently encountered in the merchandising of food products containing fats. Especially is this true in those foods in which a considerable length of time elapses before they reach the consumer. The problem involves the spoilage of fats through an oxidative process in which undesirable flavors and odors are produced. Various methods for the treatment of such foods have been developed to reduce this spoilage to a minimum. These methods include packaging in a vacuum or replacement of air by an inert gas, proper refrigeration, use of colored containers to remove harmful light rays, and the use of antioxygens.

While such mechanical means of preventing oxidative deterioration of foods as packaging in a vacuum, proper refrigeration, and the use of colored containers have proved to be of great value to certain branches of the food industry, they are of little value to the cracker baker. At the present time his main hope for the control of oxidative rancidity of crackers resides in the use of proper antioxygens.

Antioxygens are substances which, although present in only minute amounts, are able to inhibit for a time the process of oxidative deterioration. Such substances are in common use in the rubber and petroleum industries but are still in their infancy with respect to the food industry. The antioxygens used in the rubber and petroleum industries cannot be used by the food industry since they are inedible substances and so the search has continued for edible antioxygens. The knowledge that certain crude vegetable oils have excellent keeping qualities has led to a study of natural antioxygens present in vegetable oils.

Although the exact nature of most of the natural antioxygens present in vegetable oils is still unknown, several have been isolated in fairly pure form. One characteristic of most of these substances is that they contain an "OH" group and give the normal reactions of sterols. However, these natural antioxygens are not any of the common sterols since none of these possess antioxygenic activity. Just what structural characteristics cause them to have antioxygenic activity is a problem receiving considerable attention at the present time.

Table I

Samples	Antioxygenic Index
Crackers plus 0.1% Avenol......	1.2
Crackers plus 0.3% Avenol......	1.3
Crackers plus 0.5% Avenol......	1.8
Crackers plus 0.6% Avenol......	1 5
Crackers plus 1.6% Avenol......	2.6

Table II

Samples	Antioxygenic Index
0.5% Avenol added in sponge....	1.0
0.5% Avenol added in doughing up sponge...............	1.2
0.5% Alcoholic extract in sponge.	1.8
0.5% Alcoholic extract in doughing up sponge...............	1.6
5.0% Avenex added in sponge....	1.0
5.0% Avenex added in doughing up sponge...............	1.0

Table III

Samples	Antioxygenic Index
Crackers dusted on one side.......	1.3
Crackers dusted on two sides.....	1.4

That the natural antioxygens protect the oil in which they are found from rapid oxidation is a well known fact.[1,2,3] That oils containing such antioxygens, when mixed with other fats, tend to protect these fats from oxidative deterioration has also been established. For example, the author[4] found that the removal of ether soluble substances from wheat flour before baking it into crackers with lard as the shortening agent caused the crackers to have much poorer keeping quality than if they contained the ether soluble substances.

A further step in the same direction has been the use of various cereal flours or their extracts as antioxygens. Peters and Musher[5] found that, of the various cereal flours, oat flour and its extracts was the most efficient in antioxygenic activity. Oat flour was found of value in decreasing the susceptibility of ice cream to oxidized flavor by Dahle and Josephson.[6] These same authors[7] also found that parchment treated with oat flour as a sizing was effective in preventing oxidative changes in butter during storage. Rancidity development in bacon during storage was also inhibited by oat flour (Bull[8]), while Lemon[9] and his coworkers found oat flour improved the keeping quality of salt mackerel during storage.

Since crackers are subject to oxidative rancidity it was desirable to know how effective oat flour or its extracts might be in preventing this type of spoilage. Samples of oat flour (Avenex), a hexane extract of oat flour (Avenol) and an alcoholic

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extract of oat flour were obtained from the Quaker Oats Company through the facilities of Musher Foundation, Inc., and used in this study.

In the first study an attempt was made to determine whether Avenol sprayed upon crackers would inhibit oxidative deterioration and if so, how its efficiency in this respect varied with increasing percentages used. Experimental crackers were baked and, immediately, upon their removal from the oven, were sprayed with varying quantities of Avenol. The crackers were then stored in closed containers at 40 deg. C., and their deterioration in storage followed organoleptically and also by the increase in peroxide value of the shortenings contained. The antioxygenic index (the number of days samples remained sweet during storage divided by the number of days that the control remained sweet under the same conditions) gives a good idea of antioxygenic efficiency. The results of this study, summarized in Table 1, indicate the effectiveness of Avenol as an inhibitor of oxidative rancidity when sprayed upon crackers.

To determine the effect of fermentation and baking on oat flour and its extracts, crackers were made using oat products in the formula. In one series, 0.5 per cent Avenol was added when mixing the sponge and in another series when doughing up the sponge after fermentation. The same procedure was followed with a 0.5 per cent alcoholic extract of oat flour and also with 5.0 per cent quantities of Avenex itself. The results of this study are reported in Table II.

Since Avenex added to the cracker formula exhibited no antioxygenic effect, a study was made to determine if it would have any effect when dusted upon crackers just previous to the baking process. In one series crackers were dusted on one side and in another series on both sides. After baking, the excess Avenex was brushed off and the crackers stored. The results of this study are reported in Table III.

Oat flour and its extracts were found to show some antioxygenic properties by inhibiting for a time the oxidative deterioration of crackers stored at elevated temperatures. Apparently the process of cracker manufacture will destroy some of the antioxygenic properties if the material is added in the formula, especially if Avenex or Avenol are added. The alcoholic extract of oat flour retains to a considerable degree its antioxygenic properties when mixed in the formula.

The most feasible method of adding the antioxygen is by spraying the crackers with Avenol immediately upon their removal from the oven. The Avenol is absorbed by the cracker and is not detectable in the finished product if used in quantities of 0.5 per cent or less. Dusting the crackers with Avenex can also be used to improve the keeping qualities of the crackers.

Oat flour sizing on the interior of cartons (Avenized cartons), for the purpose of inhibiting rancidity, has been used commercially for more than a year and a half. Toasted wheat biscuits stored in Avenized cartons were found to retain their wholesomeness for a longer period than when stored in ordinary cartons. Further work on the use of Avenized paper in storing crackers is in progress.

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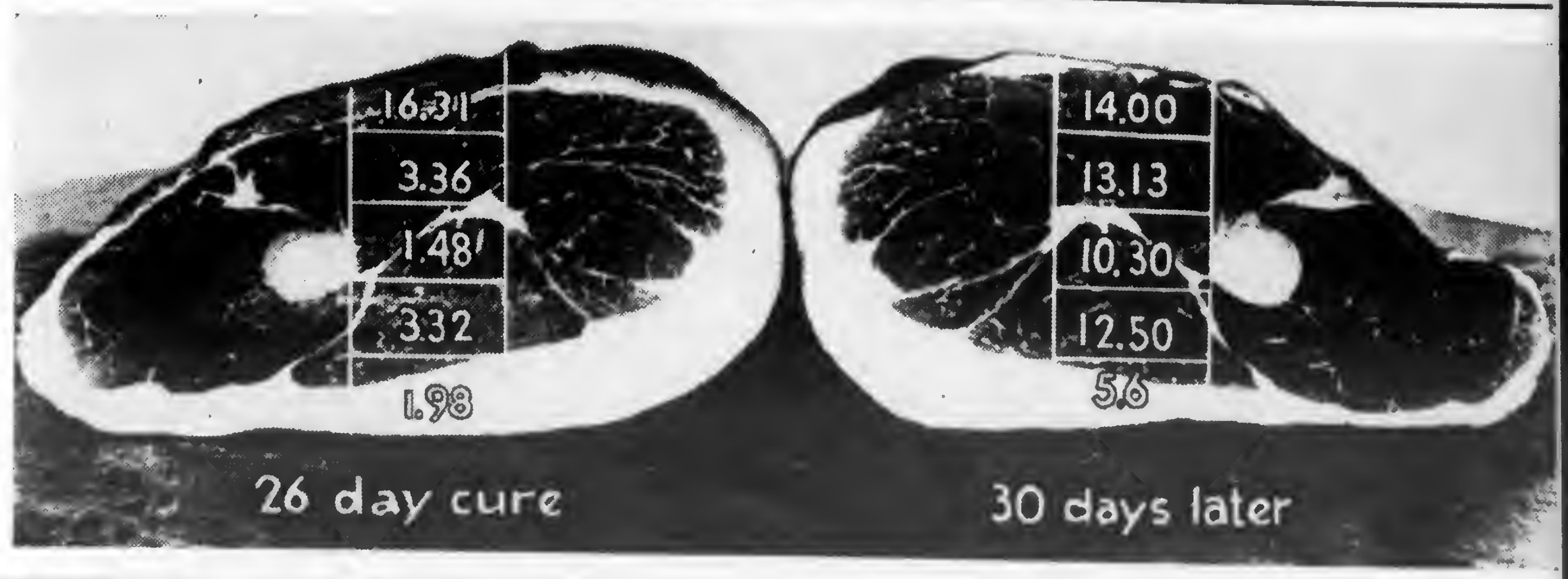
BULLETIN 355 FEBRUARY 1938



Effect of aging on distribution of salt in cured hams. Test pieces were cut out of the hams as shown by the white lines. The figures are percentages of salt in the samples at the end of the 26-day cure and 30 days later.

RATE of SALT ABSORPTION in CURING PORK

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
STATE COLLEGE, PENNSYLVANIA

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What is a Good Cure?

NUMEROUS combinations of salt, sugar and saltpeter have been recommended for pork curing. Many different recommendations produce satisfactory results. All experimenters agree, however, that sugar-cure produces the highest grade cured-pork product. No one recommends a straight salt cure (without saltpeter). Some use 12 pounds, some 10 pounds, and others 8 pounds of salt in the curing mixture for each 100 pounds of pork to be cured, with the recommendation of a two to three-day curing period per pound, regardless of formula. The result is that farm-cured pork is often under or overcured.

★

Recommendation of a cure of two days per pound for bacon is open to question. This would mean that a 10-pound bacon must be in cure 20 days. If this bacon is cut into two 5-pound pieces, should each piece be cured 10 days? The thickness of the bacon is unchanged. Some hams are deep and plump; others, of a similar weight, are shallow and flat. Some 17-pound hams show as much as three-fourths of an inch difference in thickness through the cushion directly behind the H-bone. Can salt penetrate 5½ inches of solid muscle as quickly as it can penetrate 4¾ inches?

★

Is the distance salt has to travel a more accurate basis for a curing schedule? This question prompted these investigations. Many variables enter into work of this nature, because of individual characteristics of the pork. These differences were overcome, in part, by running numerous retests over a period of three years, and by making approximately 4500 separate chemical determinations. This bulletin reports only certain of these tests; the tables represent the aggregate results of check tests.

r, Sept. 25,

THE PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION

BULLETIN 355* STATE COLLEGE, PA. FEBRUARY 1938

Rate of Salt Absorption in Curing Pork

P. T. ZIEGLER, *Professor of Animal Husbandry*, and R. C. MILLER,
Assistant Professor of Agricultural and Biological Chemistry

THE CURING FORMULA

SALT is the curing agent which functions as a preservative by either preventing or reducing the action of putrefactive bacteria. During the process of salt absorption by the muscle tissue, some of the meat juices are forced out of the meat by the difference in the specific gravity of the salt solution and the meat juices. This action causes a loss in weight of the meat that is being dry cured. The opposite occurs with pork that is cured in a brine solution.

Prolonged exposure of meat to salt action produces a dry, saline product. Incorporation of sugar in the curing mixture somewhat counteracts the astringent quality of the salt and makes the meat juicier. It also enhances the flavor of cured pork. An attractive color is as important a sales asset to cured pork as it is to the fresh meat. Color is an index to quality, but not a guarantee of it. To secure the fresh, bright pink color in cured meats, it is necessary to add saltpeter (KNO_3) or sodium nitrite ($NaNO_2$) to the curing mixture.

The mixture of different quantities of salt, sugar (brown or granulated), and saltpeter constitutes the curing formula. Any one of these ingredients is a preservative if used in sufficient amounts, but salt is the most efficient and acceptable because it also contributes to the palatability of the meat.

These investigations were conducted with the 8:3:3 formula in which the first figure represents the pounds of salt, the second figure the pounds of sugar, and the last figure the *ounces* of saltpeter. Whenever the curing mixture is applied directly to the meat by rubbing or by packing, it is designated as the Dry Cure (D.C.). When it is dissolved in water and made into a brine, it is termed Sweet Pickle (S.P.). In several instances a straight salt brine was used to test the rate of salt penetration. The salimeter, fig. 1, was used to test the strength of the sweet pickle. The salinity of a pickle is hereafter designated in degrees. Directions for making up the different strengths of sweet pickle are given in table 1.

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* Publication authorized November 8, 1937.

Both brown and granulated sugar were tested in different dry cures, as well as in the sweet pickle. Corn syrup was used in some tests on sweet pickle. Water used in making sweet pickle was boiled and the ingredients dissolved in the water while hot. This assured a water free of microorganisms and facilitated the thorough dissolution of the curing ingredients. Salimeter readings were taken at three different temperatures.

Table 1.—Amount of curing ingredients and water to be used in making different strengths of sweet pickle.*

FORMULA Pounds Salt	FORMULA Pounds Sugar	FORMULA Ounces Saltpeter	COLD WATER Gals.	COLD WATER Lbs.	DEGREE OF PICKLE BY SALIMETER 160° F	DEGREE OF PICKLE BY SALIMETER 60° F	DEGREE OF PICKLE BY SALIMETER 40° F
10	3	3	4	33 1/3	82	92	95
9	3	3	4	33 1/3	77	87	90
10	3	3	5	41 2/3	72	82	85
8	3	3	4	33 1/3	75	82	85
8	3	3	5	41 2/3	58	72	75
6	3	3	4	33 1/3	55	67	70
7	3	3	5	41 2/3	50	62	65
6	3	3	5	41 2/3	45	57	60

* Cold water weighs 8.33 pounds to the gallon. Hot water weighs 8 pounds to the gallon. Where hot water is measured, add an additional 1 1/3 pints to 4 gallons and 2 2/3 pints to 5 gallons. Seven quarts of table or fine sack salt weigh 8 pounds. One quart of corn syrup weighs 3 pounds. Either brown or granulated sugar may be used; both give the same salimeter reading.

TEMPERATURE CONTROL

Two different temperature conditions prevailed in all of these tests; they will be referred to as the controlled and the uncontrolled temperatures. Controlled temperatures refer to all pork cured under refrigeration where a mean temperature of 37° F was maintained for the dry cure and 34° F for the sweet pickle cure. Molasses barrels were used as containers for pork curing in sweet pickle; cypress boxes with perforated bottoms and open shelves were used for the dry curing.

Uncontrolled temperatures refer to pork cured in a room which averaged 47° F in the winter and 55° F in the spring and fall. The temperature was contingent upon the weather, the only control mechanism being a window.

SMOKING AND AGING

Apple wood (dried for six months) was used with corn cobs to furnish the smoke. Eighteen hours for bacon and 30 hours for ham was the usual continuous smoking period. Temperatures in the smokehouse averaged 125° F.

The aging took place under both refrigeration and room temperature. The meat was never wrapped or sacked and was hung so that it would not touch anything. No difficulty with mold was experienced, although the shrinkage was rather high.

METHODS USED IN TESTING BACON

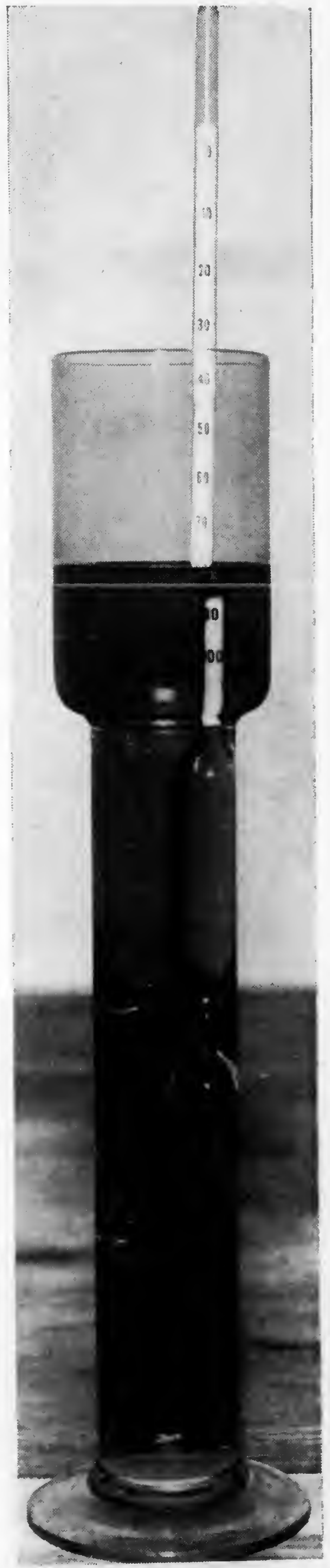
All fresh bacons were measured for thickness at a point three inches from the shoulder end. They were cured for 4, 5, 6, 7, 8 and 9 days per inch of thickness either in dry cure or in sweet pickle, after which they were removed by pairs. They were soaked in fresh water at a temperature of 60° F for 30 minutes, after which a sample was taken from one bacon and the other was smoked and aged 15 or 30 days and served as the check bacon. The samples for analysis were taken 2 inches from the shoulder end and were cut 1 inch wide, making the third inch at the place where measurements had been taken. One inch was then cut from each end of the sample, the remainder constituting true sample, fig. 2, which was divided lengthwise into three parts designated as No. 1 (the skin side), No. 2 (the center), and No. 3 (the rib side). The procedure for analysis from this point on was the same as for the ham samples. The percentage of salt was calculated on both a moisture and a dry matter basis. Since moisture is driven off in the cooking process, an analysis on a dry matter basis gives a more accurate picture of the actual salinity.

DRY SUGAR-CURING BACON

Salt absorption in bacon.—*Test A—14 pounds salt, 2 ounces saltpeter.*—The 2 ounces of saltpeter were mixed with 2 pounds of salt and this mixture was rubbed into 100 pounds of bacon. The bacons were placed in the curing

Table 2.—Results with test A; controlled temperature; bacons measured 1¾ inches.

	DAYS IN CURE 12	DAYS IN CURE 14	DAYS IN CURE 16
Days per inch in cure	7	8	9
Average salt content	5 per cent	5.25 per cent	5.4 per cent
Flavor	Very salty	Very salty	Very salty
Shrink in cure	2.5 per cent	2.5 per cent	3 per cent
Shrink, aged 30 days	6.5 per cent	6.5 per cent	6.5 per cent
Total shrink	9 per cent	9 per cent	9.5 per cent



. Fig.1.—Salimeter used to test the strength of sweet pickle.

box and the remaining 12 pounds of salt were sprinkled over the bacons as they were layered.

Test B–5 pounds salt, 3 ounces saltpeter.–This mixture was put on bacon in one rubbing. The bacon was cured on a shelf.

Table 3.—Results with test B; controlled temperature; bacon measured 1 ¾ inches.

	DAYS IN CURE 12	16
Days per inch in cure	7	9
Average salt content	3.09 per cent	3.62 per cent
Flavor	Ideal	Ideal
Shrink in cure	1 per cent	1 per cent
Shrink, aged 30 days	10 per cent	10 per cent
Total shrink	11 per cent	11 per cent

Table 4.—Results with test C; controlled temperature; bacon measured 1 ¼ inches.

	10 DAYS IN CURE
Days per inch in cure	8
Average salt content	1.34 per cent
Flavor	Very mild
Shrink in cure	None
Shrink, aged 30 days	12 per cent

Test C–8 pounds salt, 3 pounds granulated sugar, 3 ounces saltpeter.–One rubbing, using one-half ounce per pound of bacon.

Test D–8 pounds salt, 3 pounds brown sugar, 3 ounces saltpeter.–A comparison between bacon grading as lean and bacon grading as fatty.

Table 5.—Results with test D; controlled temperature.

	DAYS IN CURE 12	14	16
	Lean Bacon *		
Bacon thickness	1 ½ inches	1 ¾ inches	1 ¾ inches
Days per inch in cure	7	8	9
Average salt content	4.8 per cent	3.34 per cent	3.5 per cent
Flavor	High flavor	Ideal	Ideal
Shrink in cure	2.5 per cent	2.5 per cent	3 per cent
Shrink, aged 30 days	16 per cent	16 per cent	16 per cent
Total shrink	18.5 per cent	18.5 per cent	19.9 per cent
	Fatty Bacon †		
Bacon thickness	1 ¾ inches	1 ¾ inches	1 ¾ inches
Days per inch in cure	7	8	9
Average salt content	3.21 per cent	3.25 per cent	3.73 per cent
Flavor	Ideal	Ideal	Ideal
Shrink in cure	.5 per cent	.5 per cent	.5 per cent
Shrink, aged 30 days	9 per cent	9 per cent	9 per cent
Total shrink	9.5 per cent	9.5 per cent	9.5 per cent

* One and one-quarter ounces of the mixture used per pound of bacon.
† One and one-half ounces of the mixture used per pound of bacon.

Test E–8 pounds salt, 3 pounds brown sugar, 3 ounces saltpeter.

The ideal bacon.–These preliminary tests pointed to one ounce of the 8:3:3 curing formula as about the right amount to apply per pound of bacon. In order to get a clearer idea of what constitutes an ideal bacon, from the standpoint of salt analysis and flavor, samples of the highest grade bacons of four of the best known brands sold in the United States were analyzed. These analyses, together with analyses of the best bacons that had been produced in these trials, and others not recorded here, were used to establish the ideal bacon, the salt content of which is:

SKIN SIDE	CENTER	RIB SIDE
1 per cent	2 per cent	4 per cent

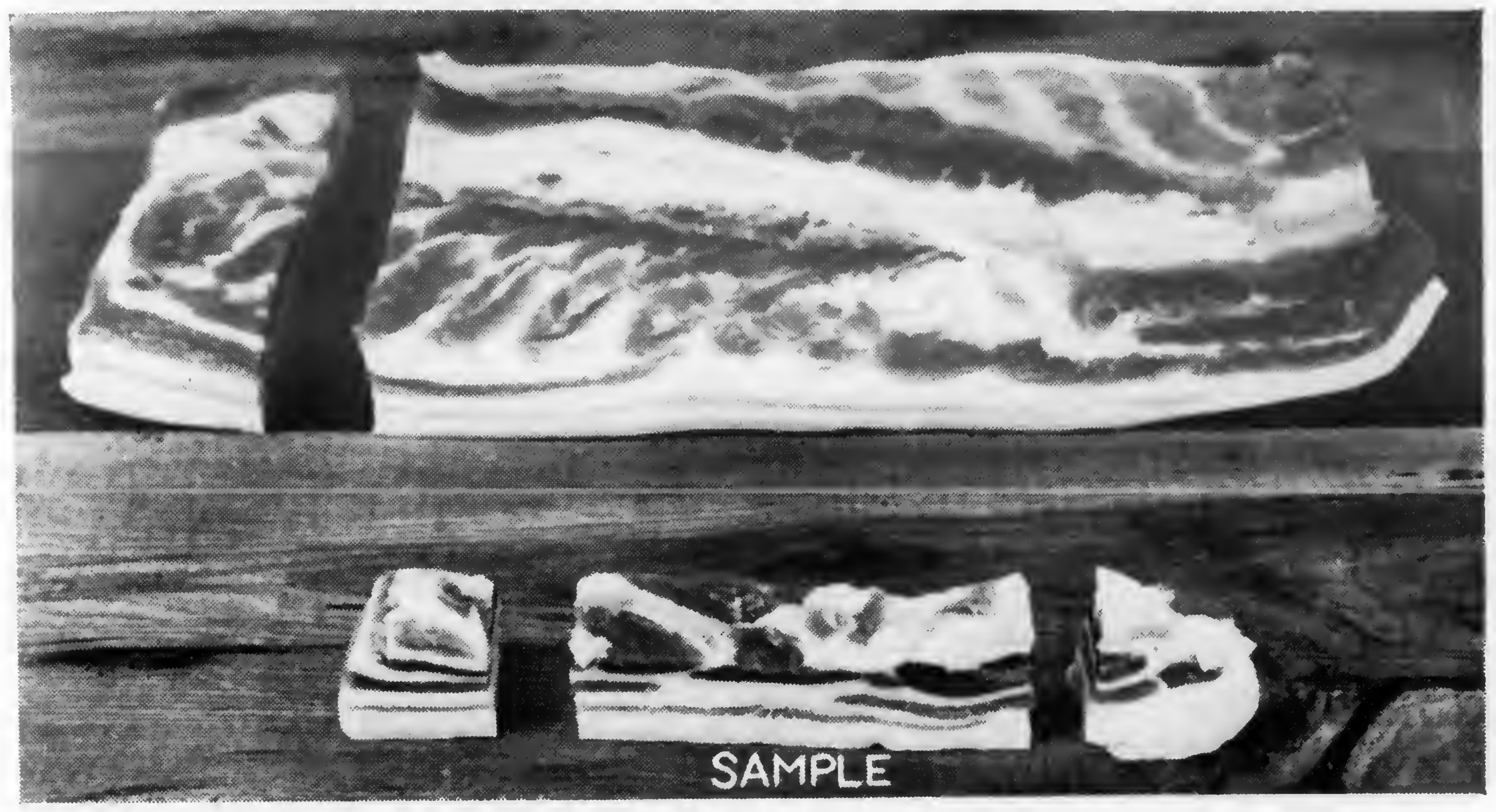


Fig. 2.—Demonstrates the method of obtaining the bacon sample.

Test F–2½ pounds salt, 1 pound granulated sugar, 4 ounces saltpeter, 1 pound of freezing pickle.–Samples were taken from a packer's vat using this cure in which several thousand pounds of bacon had reached the fortieth day in cure under a pressure of 1500 to 1800 pounds. The pressure causes the brine made by the salt and the meat juices to come to the top and seal the vat, making a convenient method of storage while the bacon is curing. The right amount of cure having been added, there is no danger that the bacon will become salty even if cured for a much longer period. The analysis of this bacon is given in table 7.

Table 6.—Results with test E; controlled temperature; used 1 ounce per pound of bacon.

	DAYS IN CURE 7	9
Bacon thickness	1 ½ inches	1 ¾ inches
Days per inch in cure	5	5
Average salt content	2.53 per cent	2.63 per cent
Flavor	Mild	Mild
Shrink in cure	4 per cent	4 per cent
Shrink, aged 15 days	12 per cent	12 per cent
Total shrink	16 per cent	16 per cent

To check further on the use of 1 ounce of 8:3:3 per pound of bacon and a curing schedule of 7 days per inch of thickness of the bacon, 14 slabs were cured, soaked one-half hour, smoked and aged 15 days. The average analysis of these slabs is given in table 7 for check X. The salt content of the rib side is rather high in most of the tests, because the period of soaking was only one-half hour instead of several hours.

Table 7.—Average analysis of cured bacon (aged samples).

	PER CENT SALT			FLAVOR
	Skin Side	Center	Rib Side	
Ideal	1	2	4	Mild
Test A	3.56	3.64	8.32	Salty
Test B	1	2.09	5.04	Mild
Test C	.58	.66	2.78	Very mild
Test D (Lean)	1.71	3.03	8.59	High flavor
Test D (Fat)	.90	3.06	5.71	High flavor
Test E	1.12	1.76	5.60	Mild
Test F	1.17	2.34	2.83	Very mild
Check X	1.19	1.80	4.65	Mild

All the trials on bacon, up to this point, were conducted in a refrigerated room averaging 37° F. Tests G and H show the results when bacon was dry sugar-cured at uncontrolled temperatures ranging from 45° to 55° F.

Table 8.—Results with test G; uncontrolled temperature.

	DAYS IN CURE	
	12	16
Bacon thickness	1 ½ inches	1 ¾ inches
Days per inch in cure	8	9
Average salt content	3.49 per cent	3.15 per cent
Flavor	Ideal	Ideal
Shrink in cure	4 per cent	4 per cent
Shrink after aging 30 days	14 per cent	14 per cent

Test G—8 pounds salt, 3 pounds brown sugar, 3 ounces saltpeter.—One rubbing, using 1 ounce of the mixture per pound of bacon.

Table 9.—Results with test H; uncontrolled temperature.

	DAYS IN CURE		
	9	10	11
Bacon thickness	1 ¼ inches	1 ½ inches	1 2/3 inches
Days per inch in cure	7	7	7
Average salt content	2.56 per cent	2.50 per cent	3.80 per cent
Flavor	Mild	Mild	Mild
Shrink in cure	4 per cent	5 per cent	3 per cent
Shrink after aging 30 days	16 per cent	16 per cent	12 per cent

Test H—8 pounds salt, 3 pounds granulated sugar, 3 ounces saltpeter.—One rubbing, using 1 ounce of the mixture per pound of bacon.

Figure 3 is a comparison of the salt content of bacons cured at controlled and uncontrolled temperatures, using 1 ounce of the 8:3:3 mixture per pound of bacon. The higher curing temperatures did not have any noticeable effect on the rate of sale penetration in the dry sugar

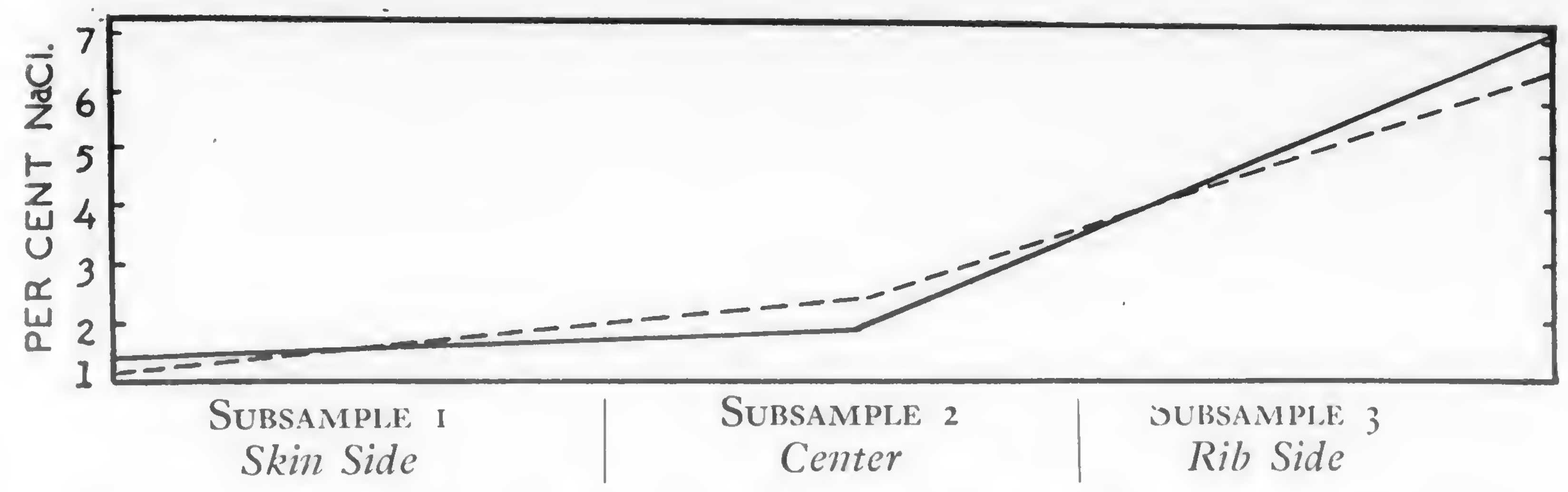


Fig. 3.—Comparison of the salt content of bacons cured at controlled and uncontrolled temperatures. The solid line represents bacons cured at 37° F; the broken line, at 50° F.

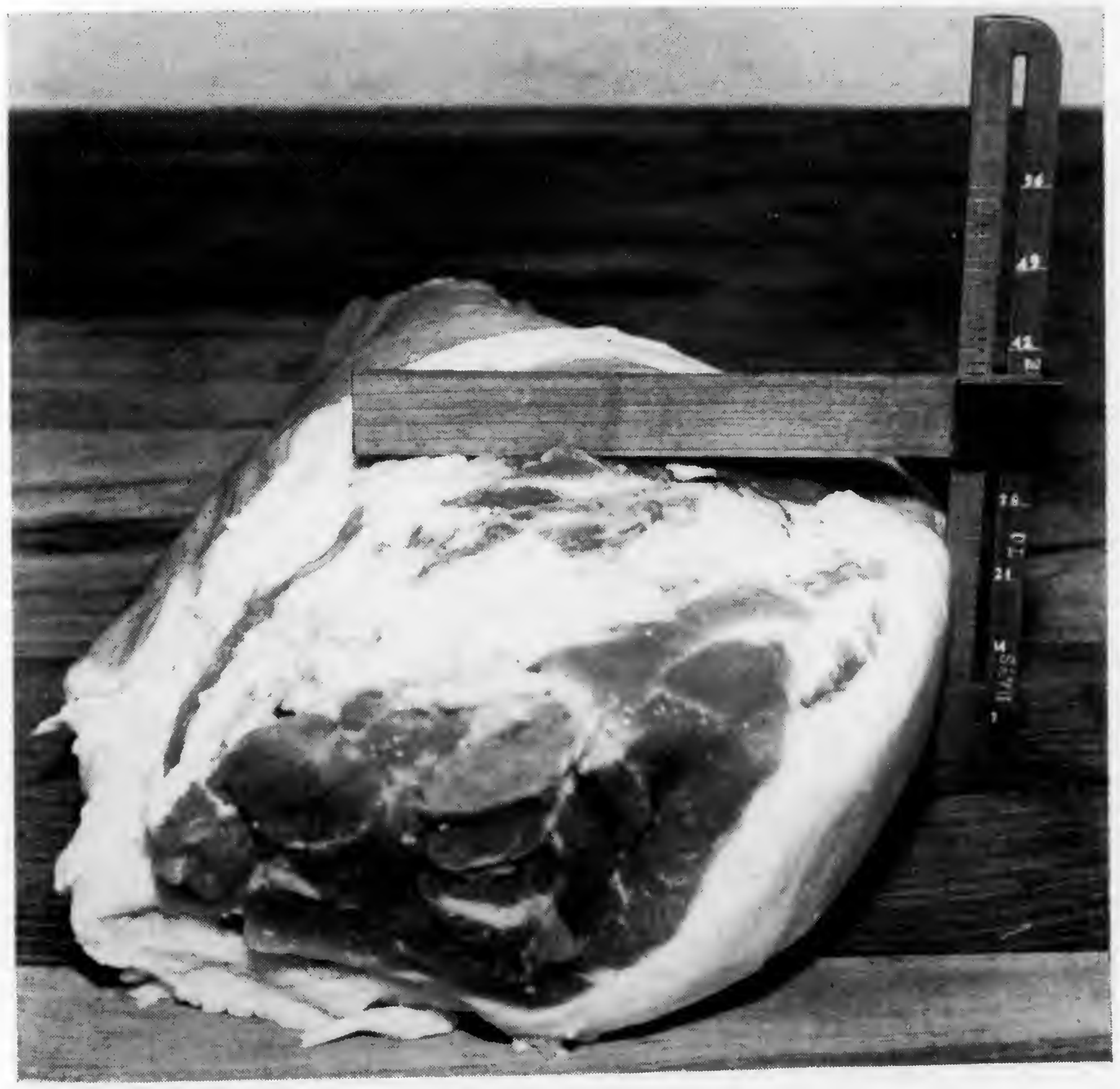


Fig. 4.—Rule with sliding right-angle arm for measuring hams.

curing of bacon. The higher temperatures did not produce any "off" flavors where the curing period was short.

METHODS USED IN TESTING HAMS

Whenever possible, both the right and the left hams from the same hog were put into cure. They were measured for thickness through the cushion directly behind the H-bone, and the measurements and green weights recorded. The measurements were taken with a rule having a sliding right angle arm, fig. 4. The rule was graduated in inches and each inch was divided into seven equal parts, to represent days. The right and left hams were removed from cure at the same time and soaked in water at 60° F for two hours to remove the excess salt from the surface. Weights were taken at the time of their removal from the cure. One ham was sampled before being smoked; the other was smoked and held for 30 or 60 days, after which it was sampled. The aged smoked ham served as the check ham. It was sampled for analysis and was given a cooking test for flavor, aroma and saltiness.

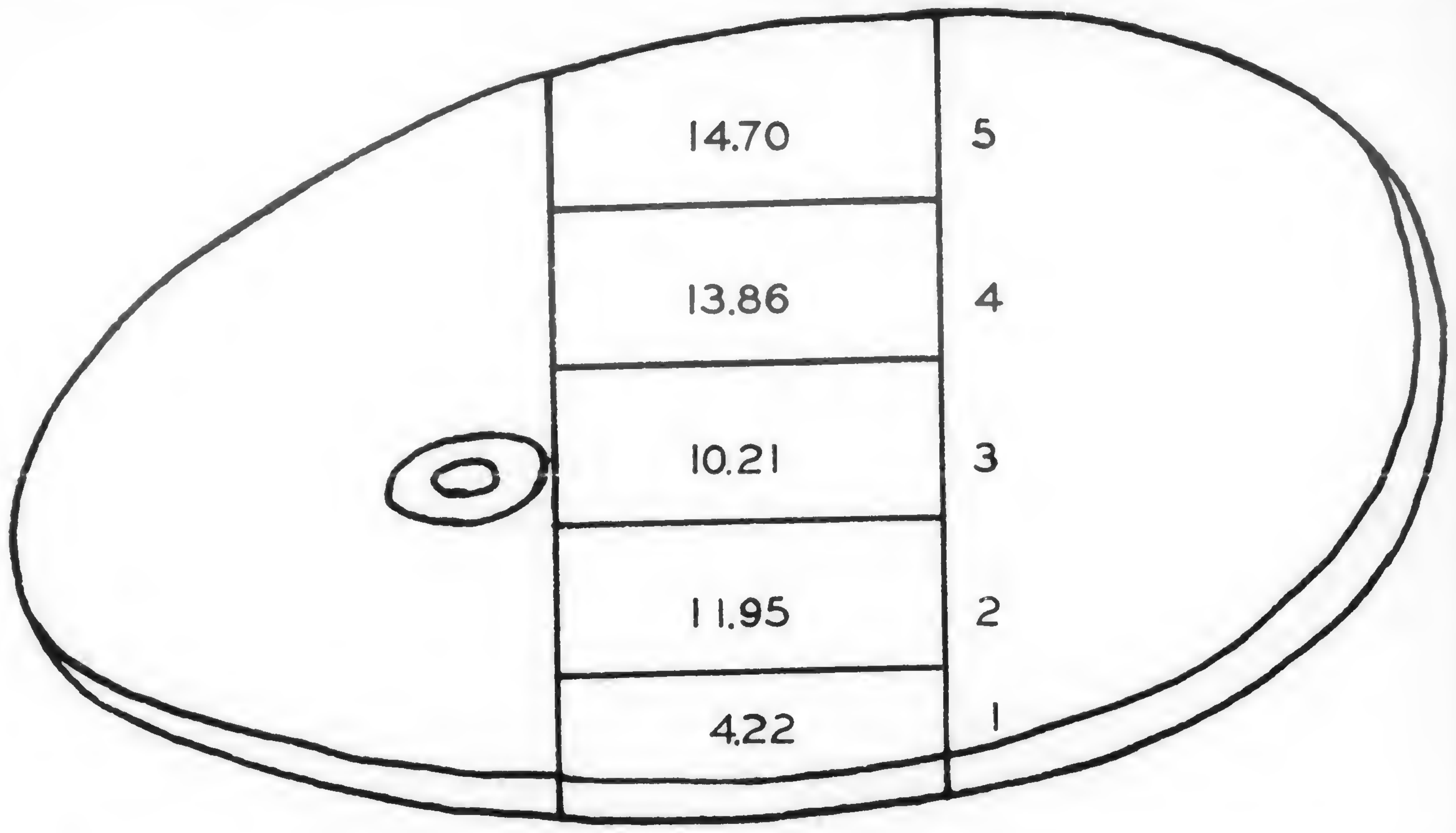


Fig. 5.—Composite of the analysis of numerous hams aged 30 days and dry-cured at controlled temperatures (37° F), showing the percentage of salt at different thicknesses.

Each ham was sampled by removing a slice 1 inch thick directly back of the H-bone *(1)* at the same location that the measurement was taken. From the center of this cut a piece 3 inches wide was removed and divided into five equal horizontal sections, fig. 5. Each of these was ground separately in a food chopper.

The sample from the outside was designated as subsample 1, and the sample from the H-bone side or inside as subsample 5. On each of these and on the three center subsamples, moisture and chlorine were determined. In this way an analysis was obtained of the entire center cross-section of the ham.

The amount of salt present in each subsample was calculated from the chlorine content, determined by the wet-digestion method as given by Davies *(2)*. A 5-gram sample of the ground meat was weighed into an Erlenmeyer flask and to it was added an excess of one-tenth normal silver nitrate, 10 cc. of saturated potassium permanganate solution, and 25 cc. of concentrated nitric acid. This mixture was boiled vigorously until all solid particles of the meat were completely digested, the average time required being about 20 minutes. Perforated glass beads were used to prevent bumping during digestion. The solution was then cooled by immersing the flask in cold water. When cool, the excess of silver nitrate was titrated with potassium thiocyanate in the presence of 2 cc. of saturated ferric alum and 10 cc. of acetone. By having a total volume of not more than 100 cc., an easily distinguishable brownish red end point was obtained without filtering off the white precipitated silver chloride from the yellow solution resulting from the digestion. A blank determination on fresh pork showed it to be so low in chlorine as not to influence the results obtained in this study, in which relatively large quantities of salt were present.

DRY SUGAR-CURING HAMS

Salt absorption in ham.—Tests were conducted on both the regular and skin-back style hams under both controlled and uncontrolled curing room temperatures.

Test I—Skin-back hams packed in plain salt, no rubbing.—The hams averaged 13 pounds and measured 4½ inches in diameter.

Table 10.—Results with test I; controlled temperature.

SUBSAMPLES	DAYS IN CURE	WIDTH OF SUB-SAMPLE, IN.	PER CENT SALT (D.M.)
1	20	.9	1.27
2	20	.9	.92
3	20	.9	1.42
4	20	.9	6.80
5	20	.9	27.70

These hams had absorbed sufficient salt in the outside 1-inch layer in 20 days (4½ days per inch) so that the remainder of the cure could take place while they were hanging and aging.

Test J—Regular hams packed in salt and saltpeter, one rubbing.—Two and one-half ounces of saltpeter were mixed with 2 pounds of salt and rubbed into 103 pounds of ham. Ten pounds of salt were poured over the hams after they had been packed in the tub. The tub had a perforated bottom.

Test J represents a curing practice that is quite common on farms. The cooking tests graded the salt content as ideal but the color was uneven and the hams were rather tough. The objection to curing hams in this manner is that they must be removed at the right time or they become too salty.

A similar test was made on hams cured in a barrel with no drainage. The brine that was formed in the barrel was sufficient to cover the first layer of hams. Every three days the hams were repacked so that each ham remained in the brine the same period. At the end of 20 days the hams were cured and graded as slightly salty. (A very good idea of the salt equalization that takes place by aging hams 30 days after they are removed from the cure is shown in the analysis of table 11.

Table 11.—Results with test J; Analysis at end of cure; hams washed but not soaked.

LBS. WT.	INCHES DIAMETER	DAYS IN CURE	DAYS PER INCH IN CURE	TEST J SUBSAMPLES				
				1	2	3	4	5
14½	5	20	4	.64	.84	.81	1.78	8.47
16½	5½	35	6½	3.91	2.22	2.05	9.37	20.22
20½	6	42	7	2.93	3.62	4.40	11.85	22.16
Check Hams—Aged 30 Days								
14¼	5	20	4	.95	4.03	3.93	7.84	11.48
16¼	5½	35	6½	3.25	9.56	8.63	12.92	15.05
21	6	42	7	1.98	5.64	6.54	11.88	14.60

Test K.–Skin-back hams were given one rubbing of a mixture of 2 pounds salt and 2½ ounces saltpeter. Four pounds of salt were then sprinkled over the packed hams. The total amount of salt used approximated 1 1/10 ounces per pound of ham.

The hams contained only 2½ per cent less salt than the hams in test J cured for the same period and averaging 1 inch less in diameter. Such methods of curing are rather inaccurate and are not to be recommended because a great deal of the salt is inactive; it lies in the bottom and is not in contact with the ham.

Test L.–This was a test on dry sugar-cured hams trimmed regular as against hams trimmed skin-back when cured under a controlled temperature of 37° F. Part of the test is reported in table 12, which is a partial reproduction of the form in which records were tabulated. The analysis for salt shows the percentage of salt computed on a dry matter basis, although the complete records also show the percentage of salt on a moisture basis and the dry matter content. This test, with others, revealed little difference in the rate of cure between skin-back and regular hams when cured with the cushion side up. Hams 1 and 2 are right and left hams, as are 3 and 4 and 1a and 2a and 3a and 4a. Hams 1, 2, 1a and 3a are check hams for hams 3, 4, 2a and 4a. The check hams were analyzed after they had aged 30 days. A similar test, in which the position of the hams was reversed, showed a decided difference in favor of the skin-backs.

These tests did not include any investigation into the proteolytic or enzymatic action that may take place in the aging process. It is concerned with the evenness of the salt distribution and its effect on the flavor as a result of the method of curing. In every instance where the analysis of the check ham or bacon which was aged was compared with the analysis of its corresponding piece just out of cure, this equalization of salt and enhanced flavor was outstanding. In other words, a freshly smoked ham is not comparable to an aged ham unless the aging is accomplished in a weaker cure over an extended curing period. This is possible and probably advisable in controlled curing cellars, but to age in the smoke is safer for the farmer.

Table 12.—Results with test L; dry sugar-cure (8:3:3 using brown sugar); curing room temperature 37° F.

	HAMS (REGULAR) 48 HR. CHILL IN CURE 10/11/33				HAMS (SKIN-BACK) 24 HR. CHILL IN CURE 11/1/33			
Ham No.	1	2	3	4	1a	2a	3a	4a
Lbs. green wt.	15.8	16.6	15.9	16.5	15.8	15.0	14.5	15.0
Wt. cured	15.2	15.8	15.2	15.8	15	14.2	13.8	14.2
Wt. aged 30 da.	13.4	14			14.2		12.8	
Shrink	2.4	2.6			1.6		1.6	
Diam. (inches)	5½	5½	5½	5½	5½	5½	5¼	5¼
Center temp. F	36°	36°	35°	35°	48°	48°	47°	47°
Days in cure	28	35	28	35	40	40	33	33
Date out	11/8	11/15	11/8	11/15	12/11	12/11	12/4	12/4
Date of check	12/8	12/15			1/11/34		1/4	
Soak (minutes)	50	60	50	60	80	80	65	65
Ounces of cure	16	16	16	16	16	15	15	15
Flavor	Good	Good			Good		Good	
Salt	Mild	Mild			Mild		Mild	
Subsample No.	*Per Cent Salt in Hams, Computed on Basis of Dry Matter*							
1	2.25	1.50	2.77	2.83	2.99	1.68	2.84	1.78
2	8.50	8.49	2.10	2.80	11.25	5.19	8.61	4.31
3	8.84	8.97	2.50	3.08	9.70	3.70	9.27	3.64
4	9.29	8.46	7.39	6.94	10.71	6.95	10.18	8.71
5	11.87	10.82	13.32	12.33	11.83	13.52	11.64	12.52

Test M.–Other trials were conducted to find the exact amount of curing mixture to apply per pound of fresh ham to produce a mild-flavored cured ham. Lack of space will not permit details of the results being included in this bulletin. It must suffice to say that 1 to 1¼ ounces of the 8:3:3 (using either brown or granulated sugar) per pound of ham, applied in three rubbings and left to cure a minimum of seven days per inch of thickness through the H-bone, produced the desired results. Granulated sugar in the mixture was more desirable than brown sugar, not only because it mixed more thoroughly and handled better but it appeared to speed up salt penetration because of its own solubility. Figure 5 is a composite of the analysis of numerous hams aged 30 days which were dry sugar-cured at controlled temperatures (37° F), using 1 ounce of 8:3:3 (8 pounds salt, 3 pounds granulated sugar, 3 ounces of saltpeter) per pound of ham, and cured seven days per inch of thickness. All the hams had good color, kept well, and graded as mild.

These tests showed that *three thorough rubbings are required to apply 1 to 1¼ ounces of cure per pound of ham.* It is not necessary, therefore, to weigh the ham or the amount of cure required for each ham. In bacon, it was found that one thorough rubbing would use 1 ounce of the cure per pound. Since this is the right amount to give the ideal cure, there is no danger of oversalting, as is the case where the cure is applied by packing.

Uncontrolled temperatures.—The tests on the dry sugar-curing of hams at 45 to 50° F resulted in a product having more desirable color and a more uniform salt distribution than the hams cured at the lower temperatures. Repeated analyses did not indicate a more rapid movement of the salt but there was a slightly greater concentration at the same speed under these higher temperatures.

Temperatures ranging between 50° and 60° F were not conducive to safe curing although hams were successfully cured at a room temperature of 60° to 65° F for 40 days. These hams had been dusted with a coarse oat flour. Unless pork has been properly handled in slaughtering (not overheated or in heat, and bled out well) and thoroughly chilled, the higher curing temperatures may prove disastrous. Of the more than 50 hams dry cured at uncontrolled temperatures, only one spoiled, which was a very low percentage when compared to the percentage lost in brine-curing at the same temperatures.

The ideal ham.—It was deemed necessary, for the sake of comparison, to set up some standard or ideal for analytical comparison. This was accomplished by sampling different well-known brands of packer's hams and testing them organoleptically and chemically. These, together with the best hams that were produced in these tests, were used in making the classification shown in table 13.

Table 13.—Per cent of salt in the ideal ham.

Subsample No.	Very Mild	Ideal	High Flavor	Salty
1	2-4	4-5	6	7
2	8-10	10-12	13	16
3	8-10	10-12	13	16
4	10-12	12-14	15	18
5	10-12	12-14	15	18

To check further on the amount of cure necessary to produce the ideal ham by the use of the 8:3:3 formula, other tests showed that while three rubbings gave the desired product when hams measured under 5½ inches, 5½ to 6½-inch hams ranging around 20 to 30 pounds required four rubbings. The curing period of seven days per inch of thickness also applied to the heavier hams. The four rubbings used about 1½ ounces of the formula per pound of ham.

CONCLUSIONS ON DRY SUGAR-CURING

(1) Dry sugar-curing was the most satisfactory method of curing pork where controlled temperatures were not available.

(2) Controlled curing room temperatures of 40° to 45° F were best adapted to dry curing.

(3) A range in temperature from 35° to 60° F did not have an appreciable effect on the rate of salt penetration.

(4) Eight pounds of salt, 3 pounds of granulated sugar and 3 ounces of saltpeter, thoroughly mixed and applied at the rate of three-quarters to 1 ounce per pound of bacon (one rubbing) and 1 to 1½ ounces per pound of ham (three rubbings for hams under 20 pounds or under 5½ inches, and four rubbings for hams over 20 pounds or over 5½ inches in thickness) produced hams and bacon that graded mild.

(5) Seven days per inch of thickness is the minimum number of days in which hams or bacon should remain in dry cure. Overcuring is impossible in this method when the brine is allowed to drain off as formed. Shelf curing is recommended.

(6) Soaking meats and allowing them to dry 24 hours before smoking produced a more uniform color than that of unsoaked meats.

(7) The major salt movement takes place from the cushion side of the ham and the rib side of the bacon. Salt absorption by the fat is slow.

(8) Skin-back hams dry-cure more rapidly than hams trimmed regular (all fat and skin left on).

SWEET-PICKLE CURE

The most widely practiced method of curing fresh hams is by the use of a sweet pickle. This is a brine made by dissolving the curing ingredients in sufficient water to make the strength of pickle desired. Picnic shoulders and shoulder butts are also generally cured in sweet pickle, but bacon is usually dry-cured. Because the salt flavor in these products must be mild and the color of the meat attractive, the curing cellar operations of packing houses are rigidly supervised. Temperature control of the curing cellar is much more important in sweet pickle curing than in the dry cure.

The large muscles of a ham are quite dense and do not take up salt so readily as the loosely muscled pork shoulders or the flat bacon sides. A great deal of ham spoilage occurs in the center, around the joint, before the salt penetrates to that point. To offset this, it is a good practice to introduce a pumping pickle (about 10 degrees stronger that the outside pickle) into the center of the ham just before it is placed in the sweet pickle. This is known as pumping a ham. A simple plunger type of pickle pump is shown in fig. 6.

The early introduction of pumping pickle into the center of the ham inhibits the development of putrefactive bacteria and the ham cures from the inside and outward as well as from the outside and inward. In several trials, the curing period of 30-pound hams which were pumped was shortened one third. The time saving depends, of course, on the amount of pump pickle used.

Both saltpeter (KNO_3) and sodium nitrite ($NaNo_2$) were used in the sweet-pickle formula. Several trials in which corn syrup replaced granulated suger showed that corn syrup is preferable from the standpoint of the color imparted to the meat. Different degrees of sweet pickle were used, table 1, and the water always was boiled before adding the curing ingredients.

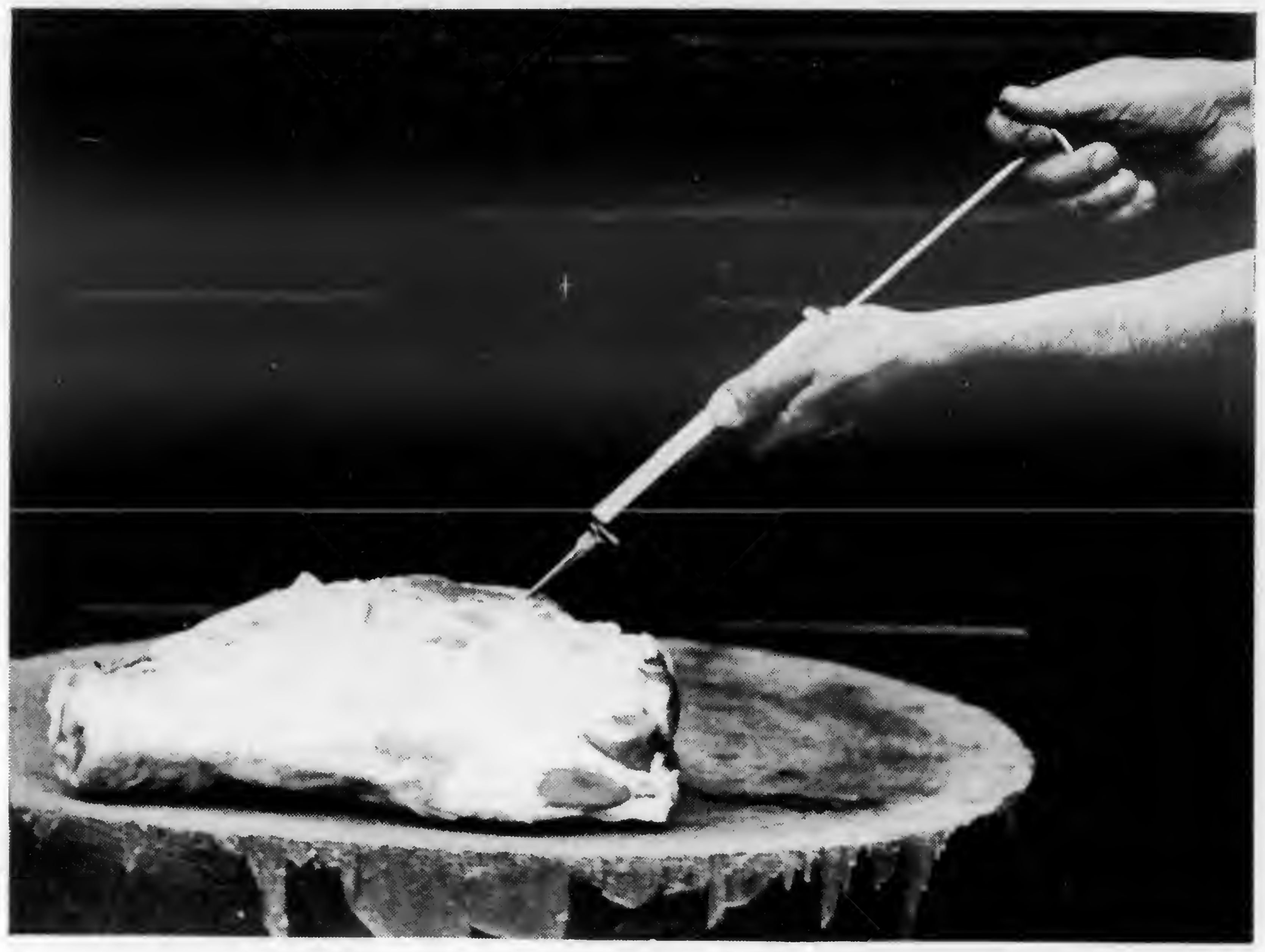


Fig. 6.—Simple plunger type of pickle pump.

Testing the strength of the pickle.—The fresh egg test has proved rather inaccurate because the egg will float in a 50° pickle as well as in a 100° pickle. The same is true of the potato test. Unfortunately, little can be said in favor of some salimeters. Of two salimeters purchased for this work, one was off 8 degrees when checked against specific gravity. The other was accurate, table 1.

Pork must be thoroughly chilled.—Removal of animal heat during the first 12 hours after slaughter is vital to the keeping quality of pork. Practically all of the complaints of pork spoilage that have come to this Experiment Station have been in connection with the brine-cure method, particularly in mild winters.

A thorough chilling, or even freezing, will assure a greater measure of success in the curing operation. Several tests were made on curing hams that had been held in a temperature of 37° F for two weeks before they were placed in sweet pickle. The hams cured in excellent shape and were far more tender than hams which had a 24 to 48-hour chill.

Where uncontrolled temperatures are used, some cracked ice should be

added to the curing pickle. With large hams, an injection of cold brine into the center of the ham before placing them in cure is advantageous.

Rarely is it advisable to cut up pork while it is still warm. The best time to slaughter hogs on the farm is during the afternoon. This provides a chance for the carcass to become thoroughly chilled overnight and makes it possible for the cutter to make a presentable job of cutting the carcass the next day. Even the best butcher will hackle a warm carcass. If the weather should turn warm and refrigeration is not available, it will be necessary to cut the carcass, place the cuts in a tub or barrel, and cover the top with cracked ice.

Table 14.—Percentages of salt in ham subsamples.

Subsample No.	Cures and Days per Inch in Each						
	75° Pickle at 34° F				75° Pickle at 50° F	75° Pickle at 34° F*	85° Pickle at 34° F
	7 days	*8 days*	*9 days*	*11 days*	*9 days*	*9 days*	*9 days*
				Out of Brine			
1	1.46	1.51	2.71	2.89	4.32	4.13	3.90
2	3.60	2.77	5.06	6.02	6.59	6.37	6.74
3	3.25	3.12	5.60	7.12	5.48	5.62	6.11
4	9.17	9.77	9.96	10.5	11.20	12.29	16.99
5	17.4	17.95	18.44	17.61	15.48	20.37	24.71
				Check—Aged 30 Days			
1	2.09	3.06	3.42	3.67	3.68	4.69	4.20
2	7.82	8.98	8.23	9.60	10.01	10.70	10.35
3	7.42	8.14	8.98	9.53	9.14	12.28	10.02
4	10.10	12.85	14.16	16.13	12.86	.14.63	14.90
5	11.91	12.08	14.12	16.96	13.98	16.58	15.82
				Check—Aged 60 Days			
1	2.71	3.10	3.62	4.02			
2	9.30	11.32	11.45	11.85			
3	9.33	10.61	12.23	12.06			
4	9.76	9.10	10.85	11.72			
5	9.92	8.49	10.41	11.98			

* Combination cure: hams rubbed with 1½ pounds of salt per 100 pounds of ham 18 hours before placing in pickle.

To test the effect of chilling warm pork in a cold sweet pickle, six hams averaging 17 pounds each were removed from hog carcasses immediately after slaughter. The inside temperature of the hams was 100° F. A sweet pickle had been made the previous day and chilled to 34° F. The warm hams were placed in the cold brine and the cure progressed at a curing room temperature of 37° F. The hams cured satisfactorily but salt absorption did not progress any more rapidly than in the chilled pork.

Another test was made on hams that had chilled in the carcass for eight hours at a temperature of 37° F. The inside temperature of the hams averaged 52° F when placed in an 85° pickle which registered 40° F. The hams averaged 15½ pounds each and were 5½ inches thick. The curing room temperature averaged 50° F. The only hams which did not spoil were the first two that were removed at the end of 28 days. These analyzed as very mild.

Combination cure.—Hams 5 inches thick, that had been removed from carcasses chilled 24 hours at 37° F, were rubbed with 1½ pounds of salt per 100 pounds of ham and left on a table over night to absorb the rub.

The next morning they were placed in a 75° pickle and cured at a room temperature of 50° F. These hams were cured 35 days or at the rate of seven days per inch. They came out of cure in excellent condition and graded as mild, table 14.

Other hams that had been chilled 24 hours were handled the same way except that the curing room temperature averaged 58° F. The brine soured in a period of three weeks. Instead of renewing the brine, all the hams were removed on the twenty-third day and hung in a refrigerator (37° F) for another 30 days before smoking. These hams, also, were in excellent condition and graded as very mild.

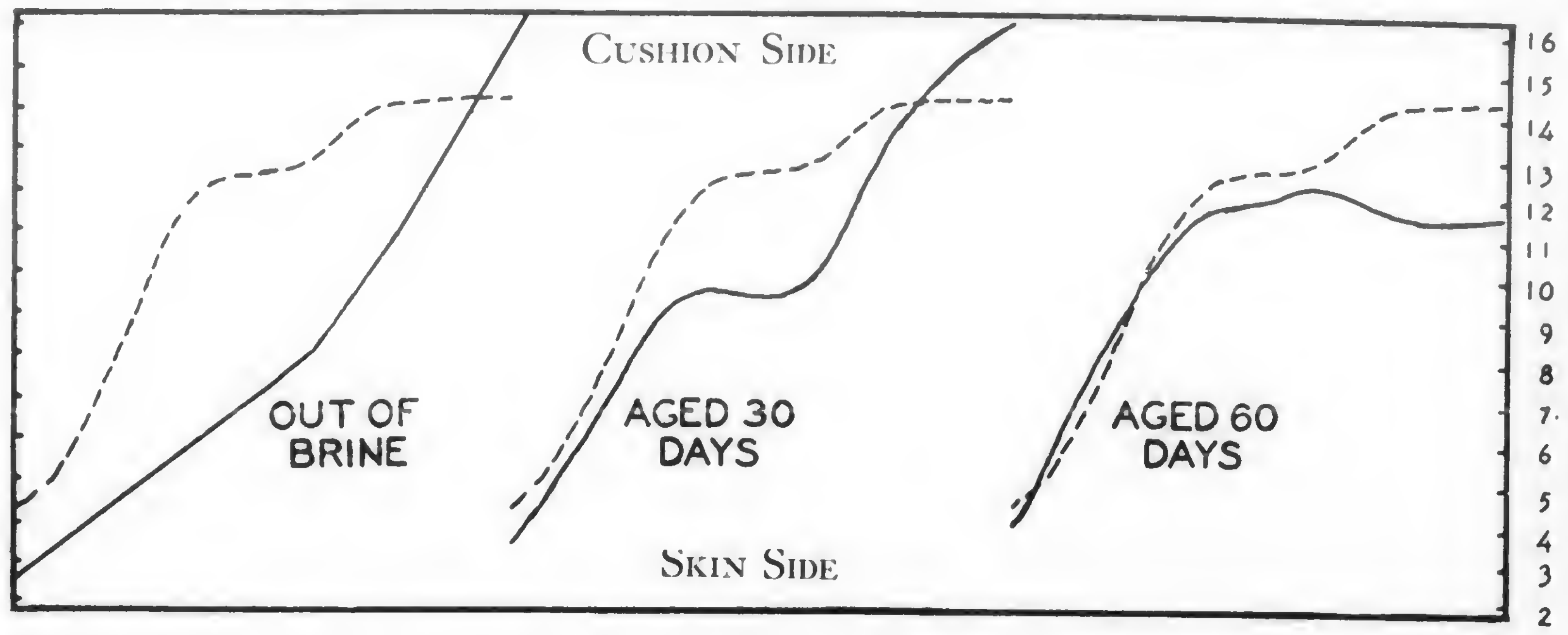


Fig. 7.—Salt analysis of hams cured in 75° pickle. The broken line represents the ideal (mild) ham; the solid line represents hams that were pickled for 11 days per inch of thickness.

The 75° sweet-pickle cure.—Eight tests were conducted involving 47 hams which were cured for a period of 7, 8, 9 and 11 days per inch of thickness in a 75° pickle at 34° F. The results appear in table 14.

These hams weighed 14 to 17 pounds and were 4½ to 5½ inches thick. A curing period of 11 days per inch produced the most satisfactory product as measured by salt analysis, flavor and keeping quality. There was a very noticeable slowing up of salt absorption by the pork that was cured in sweet pickle. It required four more days per inch of thickness to cure hams in 75° pickle than was required to secure the same results by dry curing.

Figure 7 shows the comparative analysis between the ideal (mild) ham and the hams that were pickled for 11 days per inch of thickness. Salt equalization is again well illustrated in the aged hams.

Table 14 gives the analysis of hams cured in a 75° pickle but at uncontrolled temperatures. A slight increase in salt absorption is evident, with a noticeably greater salt equalization.

The 85° sweet-pickle cure.—Table 14 also gives the salt content of a number of hams cured in an 85° pickle. The curing period was two days less per inch of thickness than was necessary in the 75° pickle. The danger of spoilage was less, particularly where a comparison of the 75° and the 85° pickle was made under uncontrolled temperatures. The analysis of hams cured at uncontrolled temperatures was practically the same as for those cured at controlled temperatures.

Sweet-pickled bacon.—Although it is not a common practice among pork processors to pickle bacon, it is a convenient way for farmers who pickle hams and shoulders to cure the side meat in the same barrel. The bacon should be placed in the top of the barrel, because of the necessarily short curing period.

Table 15.—Sweet-pickled bacon, check samples aged 30 days after removal from pickle.

DEGREE OF PICKLE	DAYS PER INCH IN CURE	PER CENT OF SALT		
		Skin Side	Middle	Rib Side
		Mild Flavor		
65°	7	.88	1.98	3.27
75°	5	.90	2.41	3.90
85°	4	.94	2.33	3.79
		High Flavor		
65°	9	1.74	2.51	3.65
75°	7	2.10	3.19	4.81
85°	6	2.43	3.11	6.42
		Ideal Flavor		
		1.00	2.00	4.00

Sixty sides of bacon were used in the tests reported in table 15. These results apply to bacon cured at both controlled and uncontrolled temperatures, since there was no noticeable difference between the two. Pickle of three different strengths were used. The curing period was for a specified number of days per inch of thickness.

While mild-cured bacon is preferred by many, the high flavor grade is by no means objectionable. The analyses in table 15 are for the check samples that were aged 30 days after they were removed from the pickle.

Table 15 may serve as a guide for those who use sweet-pickle cure for bacon.

CURING HAMS AT SUBFREEZING TEMPERATURES

Only 10 hams were used in this preliminary test; the results are presented without recommendations until further work is done. The hams were placed in an 85° pickle and cured in a room where the temperature averaged 20° F. The length of cure was 7, 8 and 9 days per inch of thickness. All of the hams were frozen solid when they came out of the pickle except the outside layer of the ham about 1 inch deep. They were hung in a temperature of 37° F for several days to thaw, after which they were soaked several hours and then smoked.

The outstanding characteristic of these hams was their tenderness.

They also had excellent flavor. The analysis of the hams just out of cure showed less than 1 per cent salt in the center but the check hams that were aged six weeks had 6 to 8 per cent salt in the center and graded as very mild.

COMPOSITE OF THREE HAMS

To determine the salt content of the entire ham, a representative ham was taken from three groups which had been cured differently. The skin, fat and lean were ground separately and analyzed.

Table 16.—Per cent of salt in entire hams.

HAM NO.	LEAN	FAT	SKIN	SUGAR	AV. SALT CONTENT
1	8.52	1.89	5.14	.95	5.99
2	10.73	3.11	3.78	.63	8.43
3	11.18	2.83	4.15		8.15

Ham 1 was cured seven days per inch in 75° pickle. Ham 2 was cured seven days per inch in dry sugar cure (1 ounce per pound of ham). Ham 3 was cured seven days per inch in dry salt cure (three-quarters ounce per pound of ham).

CURING ON THE BASIS OF DAYS PER POUND

In a study of the weight of hams versus their thickness, it was found that the following curing schedule could be used for hams:

75° pickle cure, three days per pound of ham.
85° pickle cure, two and one-half days per pound of ham.
90° pickle cure, two days per pound of ham.
Dry sugar cure (using 1 ounce per pound) cure two days per pound (minimum).
Bacon must be cured on a basis of thickness.

Table 17 shows the effect of soaking dry-cured bacons from the right and left side of four hogs for one and one-half hours. Each bacon received 1 ounce of the dry sugar-cure 8:3:3 per pound of bacon and was cured seven days per inch of thickness. The analyses show a removal of 27 per cent of the salt by soaking one and one-half hours.

Table 17.—Effect of soaking bacon for its salt content.

SAMPLE NO.	SKIN SIDE	MIDDLE	RIB SIDE
	Washed But Not Soaked		
1R	1.51	1.79	6.28
2R	2.90	2.51	8.25
3R	2.78	1.94	4.88
4R	1.50	1.43	5.69
Average	*2.17*	*1.92*	6.27
Checks	*Soaked One and One-half Hours*		
1	.97	1.34	4.50
2	2.60	2.76	7.03
3	2.22	1.85	3.14
4	.97	1.07	4.94
Average	*1.19*	*1.78*	*4.65*

The salt content of the outside of the hams was reduced 1 to 3 per cent in two hours of soaking. This is sufficient to remove the excess on the outside in order to avoid the formation of salt streaks in the smokehouse. Hams that have been overcured or cured in a strong pickle should be soaked in cool water over night.

PRACTICAL APPLICATIONS

Slaughtering should be done about 3 o'clock in the afternoon, so that the carcasses may chill over night. The dry sugar-cure, the sweet-pickle cure, or the combination cure may be used. The dry sugar-cure is recommended. Trim the hams skin-back style (it is a waste of good lard to trim them regular) and determine the weight of all the cuts to be cured.

Mix 8 pounds of salt, 3 pounds of granulated sugar and 3 ounces of saltpeter. This will cure 179 pounds of meat if used at the rate of 1 ounce per pound; or about 143 pounds if used at the rate of 1¼ ounces per pound, as is recommended for dry-curing hams. The curing ingredients are cheap; hence the amount mixed is not so important as the amount used. Rub the bacons thoroughly (this takes 1 ounce per pound). The bacons require only one rubbing, the shoulders two rubbings, and the hams three at about five-day intervals.

Place the meat on a shelf or in a box or barrel with drainage and in a room where the temperature is under 50° F. Curing will require at least seven days per inch of thickness if the hams measure about 5 inches thick, they will be ready to put into soak in 35 days. Soak them in clean cool water for several hours and then hang them in the smokehouse to drain for 24 hours.

Smoke the meat until it has the desired chestnut color and then hang it in a dry place where there is good ventilation and it is protected from flies and rodents. No covering is necessary.

If the combination cure is to be used, rub the hams with only plain salt, and let them lie overnight. On the same day, make the 75° pickle, table 1, so that it will be cool the next morning. Measure the thickness of the shoulders and hams. The hams that have been rubbed with salt are placed in the bottom of a clean, well-scalded barrel. The unrubbed shoulders are placed on top of the hams and the bacons on top of the shoulders. Pack them snugly, with the top layer of bacon skin side up. A clean board is placed on top of the meat, weighted with a clean rock. The brine is then poured over the meat until it is covered.

To secure a high-class ham, it must be cured nine days per inch of thickness. Five days per inch is sufficient for a mild-cured bacon or seven days per inch for a high-flavored bacon. Shoulders are cured the same number of days per inch of thickness (measured through the center) as bacon.

This pickled meat must be placed in a cool cellar at a temperature between 35° and 45° F, and certainly not over 50° F. The meat may spoil if the hog was not bled out properly or thoroughly chilled. In a book, or on a card which is tacked on the barrel, place the dates when the bacons shoulders, and hams are to be taken out of pickle. The procedure thereafter is as indicated for the dry cure.

CONCLUSIONS ON SWEET-PICKLE CURE

(1) The rate of salt absorption was dependent on the salinity of the brine. A curing period of 11 days per inch through the H-bone gave the desired results when a 75° pickle was used. Nine days per inch was satisfactory for the 85° pickle.

(2) There was no difference in the rate of salt absorption between regular and skin-back hams in sweet pickle.

(3) There was slightly more rapid salt penetration at 45° to 50° F than at 34° F.

(4) The higher temperatures produced improved color as well as more pronounced salt equalization.

(5) The 65° and 70° pickles proved more desirable for the longer cures under controlled temperatures because they afforded a means of storage, and at the same time salt equalization was taking place to a marked degree.

(6) A mild ham at the end of the cure became a high-flavored ham in six months. This was due to shrinkage and subsequent greater salt concentration.

(7) There was very little difference in the shrinkage of pork cured by the dry and the sweet-pickle methods at the end of a 60-day aging period. Sweet-pickle-cured hams and bacon gained an average of 5 per cent in weight in pickle, whereas the dry-cured meats lost 5 to 7 per cent in cure. Sweet-pickle meats lost 5 per cent in smoking, compared to 2 per cent for dry-cured meats. After 30 days out of smoke, the sweet-pickle meats showed a 9 per cent shrink for ham and 14 per cent for bacon, as against 11 per cent for dry-cured hams and 15 per cent for dry-cured bacon.

(8) A higher and more even flavor was secured by aging bacon two weeks and hams four weeks after smoking.

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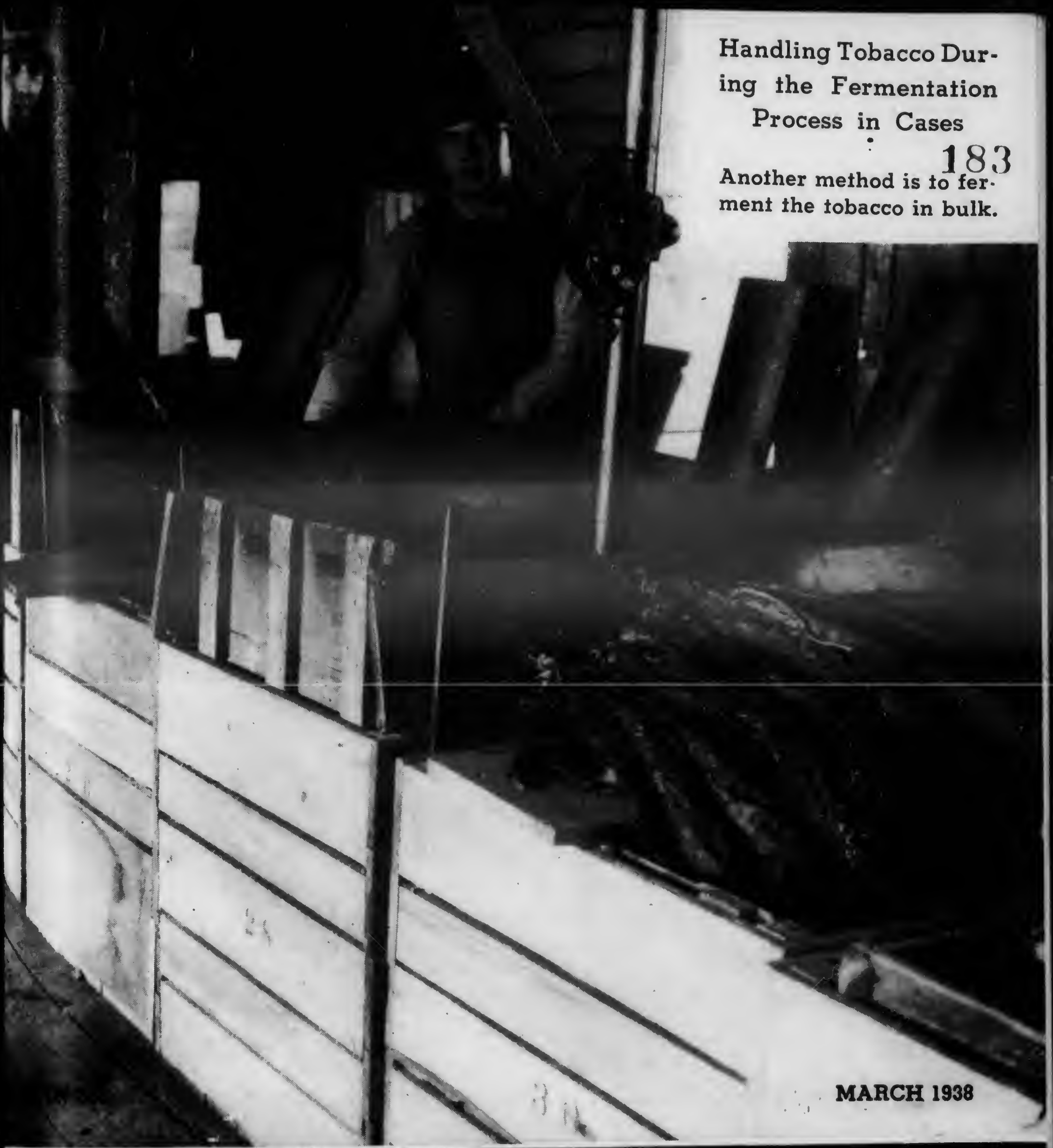
(1) **Moulton, C. R. 1929.** Meat through the Microscope. University of Chicago Press, Chicago, Ill., 112 pp.

(2) **Davies, W. L. 1932.** The determination of chlorine in dairy products and biological material. *Analyst* 57:79-85.



Handling Tobacco During the Fermentation Process in Cases

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Another method is to ferment the tobacco in bulk.

MARCH 1938

Studies on the FERMENTATION OF TOBACCO

Microflora of Cured and Fermenting Cigar-leaf Tobacco

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
STATE COLLEGE, PENNSYLVANIA

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PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION

BULLETIN 356* STATE COLLEGE, PA. MARCH 1938

Studies on the Fermentation of Tobacco

1. The Microflora of Cured and Fermenting Cigar-leaf Tobacco

J. J. REID, *Assistant Professor of Bacteriology,* D. W. MCKINSTRY, *Graduate Scholar in Bacteriology,* and D. E. HALEY, *Professor of Soil and Phytochemistry*[1]

PROCESSING of tobacco of the cigar-leaf type involves certain periods of fermentation. Reactions of an exothermic nature characterized by oxygen uptake and the evolution of carbon dioxide, ammonia and other volatile substances take place during this process. The course of fermentation appears to be affected profoundly by variations in composition of the leaf which are due to differences in environmental conditions, among which may be mentioned the soil, season and cultural practices on the farm. Great losses to the industry are occasioned by the failure of certain crops to undergo a satisfactory fermentation in the warehouses of the manufacturer.

Although the fermentation of tobacco has been the subject of study for more than 50 years, little exact knowledge exists as to the different factors involved. Improvements in methods employed by the manufacturer have been to a great extent the results of empirical information acquired in the industry rather than the result of scientific study of the problems involved.

Three theories have been advanced to account for the chemical changes occurring during the fermentation process by workers who have studied the problem. The first theory advanced maintained that the reactions were purely of an oxidative character and were not catalyzed by enzymes. That the fermentation of cigar-leaf may be explained in this manner is no longer the belief of those familiar with the process, but the literature of recent years

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[1] The assistance of Otto Olson, U. S. Department of Agriculture, is gratefully acknowledged.

on the subject of the fermentation of other types of tobacco implies acceptance of this theory. The second theory advanced ascribed the chemical reactions as due to the activities of microörganisms. Although 50 years have passed since the inception of this theory, the support accorded it in recent years has been vague and somewhat contradictory. The third theory attempts to account for the chemical reactions during fermentation on the basis of catalysis by plant enzymes, and this appears to be accepted by the majority of those engaged in the processing of cigar-leaf tobacco at the present time. Its supporters have vigorously attacked the bacterial theory and left it but few adherents.

The present study was undertaken in an effort to reconcile some of the divergent views of previous workers and determine if possible the nature of the fermentation of cigar-leaf tobacco and the mechanism involved.

REVIEW OF LITERATURE

The belief that the changes occurring in fermenting cigar-leaf tobacco may take place in the absence of enzymatic activity is found frequently in the literature on the subject. This theory was advanced by Nessler *(19)*. Although many workers since that time have hesitated to support this non-biological theory, others have found it—in the face of a lack of sufficient scientific evidence—convenient to do so. An unbiased analysis of the observations of many of the investigators in this field tends to indicate that certain of the chemical reactions taking place in the fermentation process are indeed uncatalyzed by enzymes.

Koller *(12)* inoculated tobacco with a yeast infusion in an effort to hasten the fermentation. Fesca *(5)* compared the tobacco fermentation to that of ensilage. The same year Schloesing *(22)* concluded that the process was somewhat similar to that taking place in fermenting manure. These early suggestions relating to the role of microorganisms in the fermentation of tobacco were followed by the observations of Suchsland *(25)* in 1891, who reported the isolation of bacterial cultures from tobacco undergoing fermentation. He further claimed that these organisms were the principal agents involved and that a certain quality could be imparted by artificial inoculation of tobacco. Within the following 10 years Miciol *(18)*, Davalos *(4)*, Behrens *(1)*, Vernhout *(26)* and Koning *(13)* published in general support of the bacterial theory advanced by Suchsland.

Loew *(14)* in 1899 published the first of a series of three papers in which he vigorously attacked the bacterial theory of Suchsland *(25)* and offered the theory that plant enzymes were wholly responsible. The bitter attack of Loew placed the bacterial theory in ill

repute for a period of 25 years, and the influence of this series of publications may be seen in practically all subsequent publications on the subject. Boeckhout & Ott de Vries *(2)*, Jensen *(8, 9)*, Cohen & Jensen *(3)* and Smirnov *(24)* published in confirmation of the work of Loew, and many other research workers accepted his conclusions and based the results of their own investigations upon his theory. The conclusions of Johnson *(10)* admit the possibility of the plant enzyme mechanism.

In 1925 there appeared two publications, Schmidt *(23)* and Joergensen *(11)*, lending support to the bacterial hypothesis of Suchsland. More recently Johnson *(10)* and Giovannozzi *(6, 7)* have presented evidence suggesting that microörganisms may play an important role in the fermentation of tobacco.

The following publications are deserving of particular consideration in connection with the investigation reported in this paper:

Vernhout *(26)* studied cured tobacco leaves and leaves representing various stages of fermentation. He found two distinct types of bacteria consistently present. These proved to be species of *Bacillus* which, according to the description of the author, would be classified today in the *subtilis–mesentericus–vulgatus* group of this genus. He described these as Bacterium A and Bacterium B, of which A grew at somewhat higher temperatures than did B and was slightly less proteolytic. The description of A more closely conforms to that of the *subtilis–mesentericus–vulgatus* group. The author believed B to be more important in the earlier stages, and A to be the important form in later stages of fermentation.

Schmidt *(23)* investigated the fermentation of German and certain other tobaccos, particularly some which were grown in Ohio. He found that the microflora present on the tobacco passed through selective changes as the fermentation proceeded. The particular types of bacteria present during the fermentation were found to vary with the quality of the tobacco, and different methods of fermentation resulted from different types of organisms. On Ohio tobacco of good quality he found only bacteria of the *subtilis–mesentericus–vulgatus* group, while with some of poorer quality he found various cocci. Members of the genus *Clostridium* were found on tobacco of very poor quality which underwent an unsatisfactory fermentation. Short rods, fluorescent bacteria and large spore-formers of the *mycoides* and *megatherium* types were found on some samples.

Johnson *(10)* reported a study of the fermentation of Wisconsin and other cigar-leaf tobacco. He isolated and studied in pure culture species of fungi of *Aspergillus, Penicillium* and*Oöspora,* two unidentified cultures of bacteria and a species of *Staphylococcus.* He treated samples of tobacco with chloroform, mercuric chloride, acetone, toluene, beta naphthol, silver nitrate and heat in an effort to disinfect the samples. He found that all of these treatments, with the exception of silver nitrate, completely checked thermogenesis. The silver nitrate treatment reduced theromogenic activity. He concluded that in the case of this disinfectant he was able to inhibit bacterial activity without seriously affecting enzymatic activity. In the other cases he concluded that he had destroyed both. He reported that a temperature of 85° C for one hour would completely sterilize the samples. Pure-culture inoculation of such heat-treated tobacco showed that the bacterial species isolated were incapable of inducing thermogenesis although species of fungi induced heating. He concluded that microörganisms, especially fungi, may play a role in the fermentation or processing of certain types of cigar-leaf tobacco although such organisms may not necessarily be essential to fermentation.

The attack of Loew *(14, 15, 16)* upon the bacterial hypothesis of Suchsland has been an important factor in all research on this subject in recent years. From a bacteriological standpoint the data of Loew are far from satisfactory, the conclusions being rather arbitrary considering the data presented. Loew indicted the bacterial theory upon a number of grounds. He maintained that direct microscopic observation of fermenting leaves failed to reveal a sufficient population of microorganisms to account for the profound chemical changes of fermentation. He further claimed that the moisture content was insufficient to permit growth of bacteria, and that the expressed juice of the cured or fermenting leaves would remain sterile. Loew claimed that the cellulose of the leaf would of necessity be vigorously attacked if bacteria were the responsible agents and that evidence of cellulose attack in a satisfactory fermentation was lacking. In order to account for the enzymatic activity which he found to be associated with fermentation, Loew postulated the plant enzyme theory. That this activity is entirely due to plant enzymes has been challenged by the authors in previous publications *(17, 20, 21)*.

TECHNIQUE

For the purpose of this study cured tobacco was obtained from growers and manufacturers of Pennsylvania, Wisconsin and Ohio cigar-leaf, and fermenting and fermented tobacco was obtained from manufacturers engaged in the processing of these crops. Samples rated by manufacturers as good, fair and poor were selected and studies were conducted under laboratory and plant conditions.

Two methods of sampling for bacterial assay were practiced throughout the course of the investigation. One method consisted in cutting 100 sq. cm. of surface area from the leaf, the sample consisting of two 40-sq. cm. and one 20-sq. cm. portion taken from different locations on the leaf. The other method consisted in the removal of a weighed sample, usually 2 grams, although in some cases larger samples were taken in order to procure a representative sample.

In the preparation of suspensions of the samples for bacterial counts and for determining enzyme activity, two methods were also used throughout the course of the study. In one case the leaf material was dried at room temperature and ground in a sterile mortar. The second method was an analysis of the moist leaf, as follows: The moist sample was placed in a sterile 100-ml. water blank containing several glass beads and allowed to stand for 45 minutes. This was followed by a three-minute period of vigorous shaking. Appropriate dilutions for the assay were made in sterile water blanks at this point. Comparison of the results obtained by the use of the two methods will be found in the discussion of experimental results.

Counts of microorganisms were made by the plate method and in many instances this information was supplemented by direct microscopic counts, using the Petroff-Hauser chamber. Preliminary investigation showed beef-extract-peptone agar, adjusted to a pH of 6.8, to be a suitable substrate for use in making the plate counts of bacteria. Media containing various concentrations of tobacco infusion were investigated but seemed to offer no advantage when compared with the common laboratory media. Several energy and nitrogen sources were introduced in preliminary trials but these additions failed also to improve the substrate. In efforts to detect the presence of gram-negative organisms a suitable concentration of crystal violet was added to the beef-extract-peptone agar medium.

For the purpose of determining the relative numbers of fungi on various samples, dilution plates poured with glucose-peptone-acid-agar, adjusted to a pH of 4.5, proved best adapted.

Various incubation temperatures were resorted to in the determination of bacterial numbers. Parallel plates were poured and incubated at temperatures of 20, 30, 37, 45 and 55° C. Plates were occasionally incubated under anaerobic conditions, using potato tissue to remove the oxygen from the atmosphere of the anaerobic jars. Incubation time varied with temperature; a period of 24 hours was found sufficient for temperatures of 37° C and higher, and periods ranging from two days to four days proved adapted to the lower temperatures. All acid-agar plates were incubated at room temperature and readings made on the third and fifth days.

On a number of occasions a pasteurization treatment was resorted to in an effort to destroy all vegetative forms in the sample and to stimulate the germination of bacterial spores. This was accomplished by holding the water suspension of the sample at 80° C for 15 minutes. As this treatment invariably resulted in the elimination of all fungi and gram-negative organisms it was assumed that all vegetative forms were destroyed in the procedure.

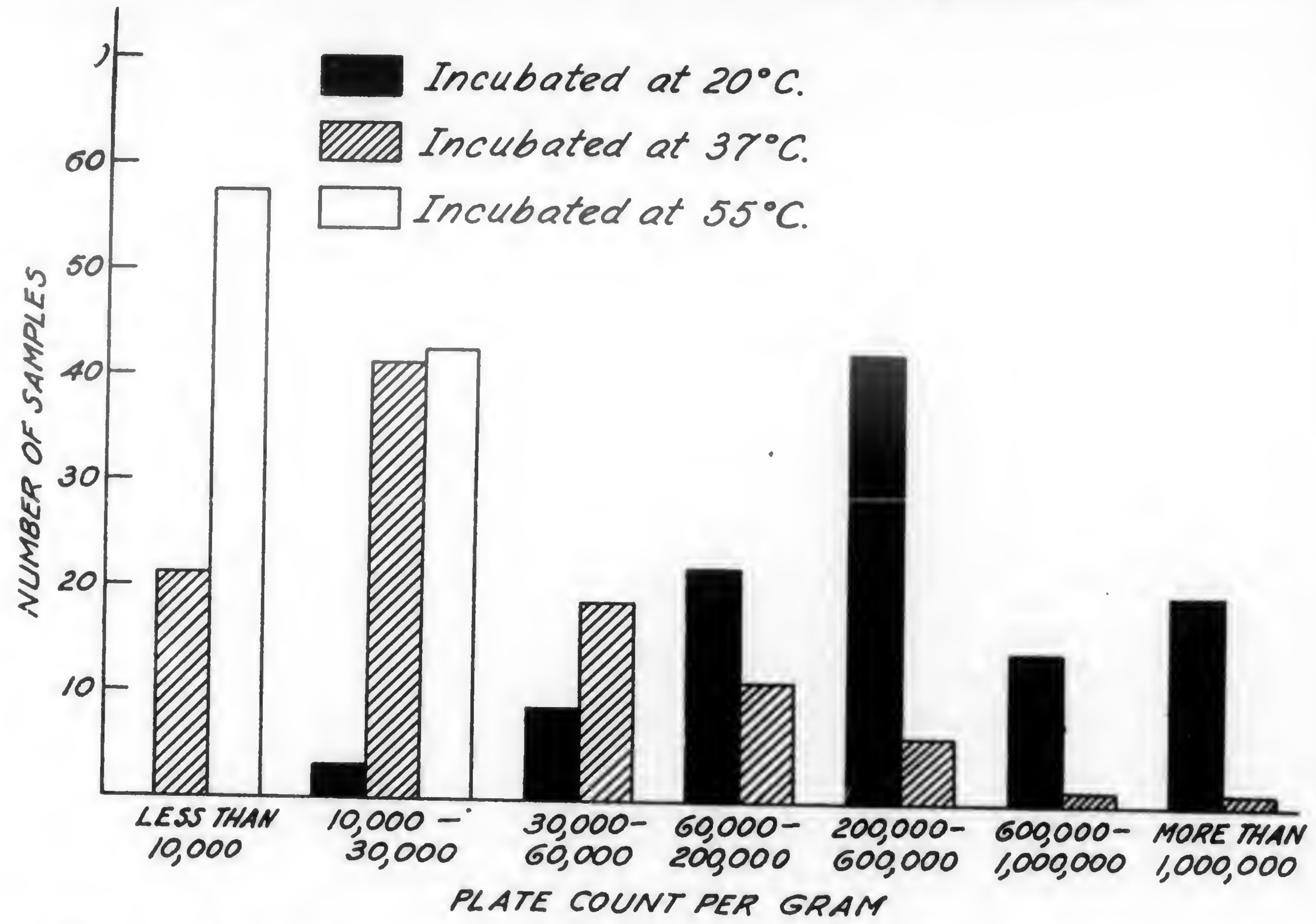

Fig. 1.—Aerobic reproduction of bacteria on nutrient agar incubated at different temperatures, based on studies involving 100 samples of cured cigar-leaf tobacco.

The usual laboratory methods were employed in the pure culture studies of isolations made in the course of the investigation.

Determinations of pH were made colorimetrically or by the use of the glass electrode as noted. Moisture determinations were based on the loss of weight on oven drying at 103° C for a period of four hours.

EXPERIMENTAL RESULTS

Flora of the cured leaf.—During the course of these investigations a great many samples of freshly cured cigar-leaf of the 1936 and 1937 crops of Pennsylvania and Wisconsin tobacco were examined. An analysis of the numbers of bacteria found upon the lamina of 100 typical samples is presented in fig. 1.

Temperature of incubation is an extremely important factor in the determination by plate count of the number of bacteria present. All of the 100 samples showed less than 30,000 viable organisms per gram when the plates were incubated at 55° C. Incubation at 37° C gave somewhat higher counts, while incubation at 20° C gave still higher counts. Approximately 70 per cent of these samples gave values greater than 200,000 per gram at the latter temperature of incubation. No sample examined was found to show less than 15,000 viable bacteria per gram with plate incubation at 20° and none showed less than 6,000 per gram at 37° C. Incubation at 45° resulted in counts greater than those obtained at 55° but lower than those obtained at 37° C. Likewise counts at 25° and 30° ranged in order between those obtained at 20 and 37° C. Counts at 25, 30 and 45° C are not indicated in fig. 1.

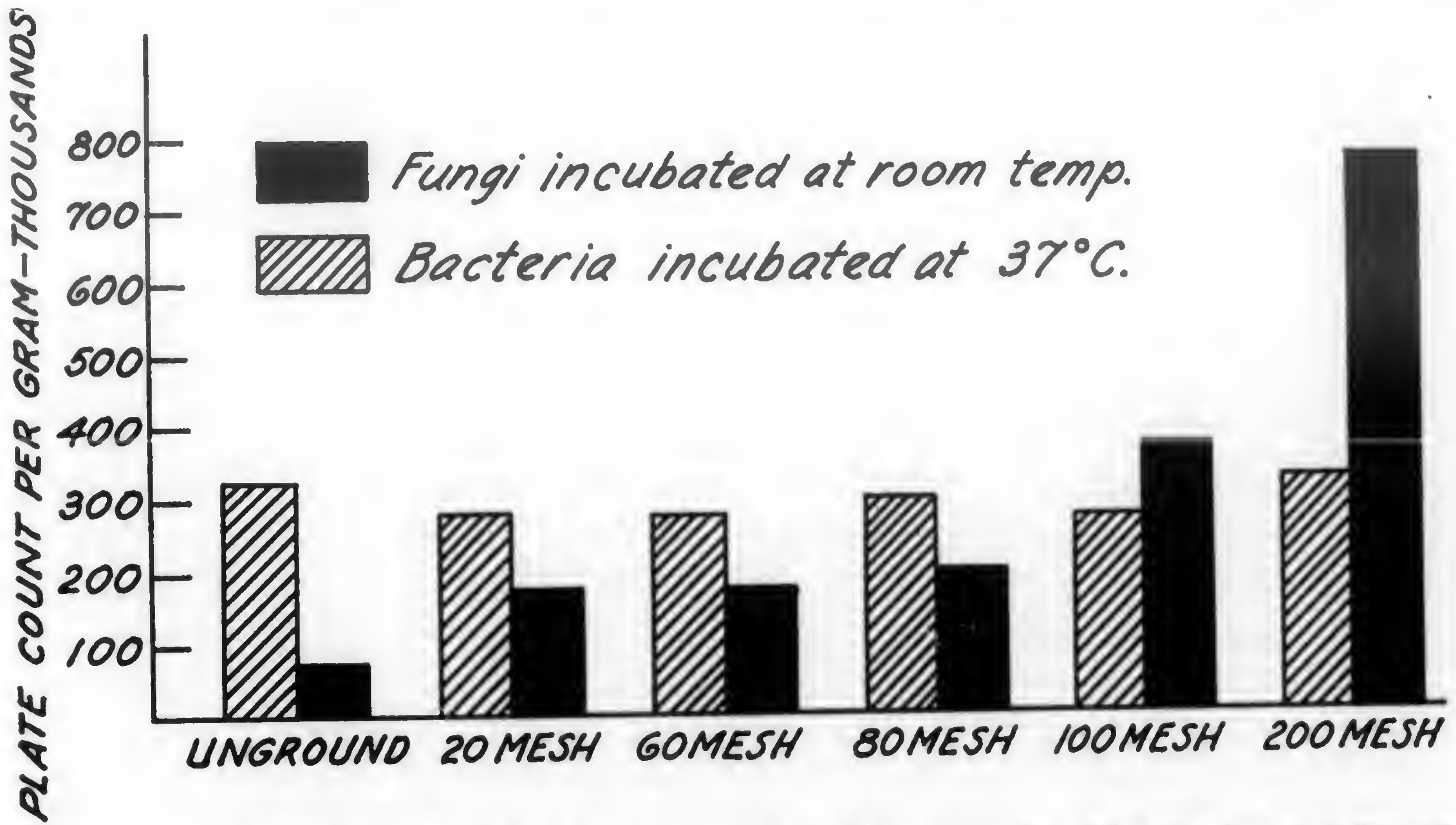

Fig. 2.—Effect of particle size upon the plate counts of bacteria and fungi found on cured cigar-leaf tobacco.

Additions to the standard beef-extract-peptone agar used in preliminary trials and differents methods of sampling failed to show significant differences in counts. In an effort to determine the most suitable method for the preparation of the material under investigation, plates were poured from samples, some of which had been finely ground and others suspended without grinding. The results, typical of the many tried, are shown in fig. 2. No significant effect on the number of bacteria was noted but the effect on the plate count of fungi was marked in every instance, owing to the fragmentation of viable mycelia. Counts of fungi on cured cigar-leaf ranged as a rule between 10,000 and 100,000 per gram when the suspension was prepared without grinding.

Of more significance were the types of organisms found upon the cured leaf. Bacteria present on the plates were largely spore-formers and cocci; fungi present represented for the most part members of *Aspergillus, Penicillium, Mucor* and *Rhizopus*. Many platings of tobacco leaves revealed the presence of gram negative forms, types that were greatly reduced in number during the curing process. Crystal violet plates inoculated with dilutions of cured tobacco usually were overgrown with fungi, indicating the small numbers of gram negative bacteria surviving the curing process. This is a reflection of the composition of the substrate inasmuch as bright cigarette-leaf tobacco was found to carry a significantly large number of gram-negative rods following the much more drastic flue-curing process.

Having established the fact that a considerable part of the population of the cured cigar-leaf consisted of spore-forming bacteria, the difficulties involved in making reliable determinations of numbers of viable microörganisms became obvious. Plate counts of microörganisms reflect the suitability of the substrate employed and the conditions of incubation rather than the exact number of viable cells on the material under examination. In dealing with spore-formers the problem becomes further complicated. Viable spores do not necessarily germinate upon the plate although the substrate may be suited to the reproduction of the vegetative cells of the species. Nevertheless, recourse was had to the direct count and likewise to the use of microtome sections in an effort to establish the total number of bacterial cells present on the cured leaf. Such counts were to prove of particular value when studied in connection with direct counts made on the fermenting leaf.

Ground material could not be used for this purpose as the artifacts rendered a count impossible. It was found, however, that if the same procedure was followed as was used in the plating of unground samples a fairly satisfactory count could be made. Such preparations usually showed from 400 million to 800 million cocci and spores per gram of cured tobacco. Rods were seldom seen. It would appear, therefore, that some reproduction must have taken place on the leaf, either during the growing season or during the period of curing.

Contrary to the observations of others, none of the samples examined was found to be sterile. When compared with examinations made of green leaves it was seen that a certain amount of selection had taken place. The flora of the green leaf, including as it does most of the common saprophytes of the soil, had become, in the case of the cured leaf, a flora consisting of cocci, spore-forming bacteria and those fungi commonly termed molds. Some reproduction had apparently taken place; first because of the large number of cocci and spores found by direct observation, and second because of the presence of fungus mycelium upon the leaf.

Flora of the fermenting leaf.—Suchsland *(25)*, Vernhout *(26)*, Schmidt *(23)*, Johnson *(10)* and others have reported the presence of large numbers of microorganisms upon fermenting cigar-leaf tobacco. These observers have not been in complete accord as to the type or types of microorganisms multiplying during this process. Aerobic spore-forming bacteria, cocci and fungi have each been looked upon as causative agents of the fermentation or aging of this type of tobacco. On the other hand, the proponents of the plant enzyme theory have denied the presence of any considerable number of living microöranisms on the fermenting leaf and have stated that reproduction of such forms was impossible under the conditions of satisfactory fermentation. In an effort to account for these conflicting observations attempts were made in the laboratory to carry out the fermentation of tobacco of various grades over wide ranges of moisture-oxygen-temperature relationships.

It soon became apparent that fungi, cocci and spore-forming bacteria were able to multiply rapidly upon the cured leaf of average quality if proper conditions were provided to satisfy their individual needs. Low moisture and large amounts of oxygen provided suitable conditions for the development of fungi; a slight increase in the moisture content provided a satisfactory environment for the rapid growth of cocci and aerobic spore-formers; anaerobic conditions, brought about in various ways, resulted in the activities of members of *Clostridium* and the anaerobic respiration of the facultative cocci and members of *Bacillus*. Contrary to the observations of Johnson *(10)*, only the aerobic development of cocci and aerobic spore-forming bacteria was found to be associated with a satisfactory fermentation. The judgment of the authors was not relied upon in this connection; samples were submitted to leaf experts in the industry for their decision as to whether or not the fermentation might be termed satisfactory.

Fungi developing upon the cured leaf were usually found to be members of the genera *Aspergillus* and *Penicillium*. Occasionally the development of members of the *Mucorales* was noted upon tobacco of good quality. Growth of the fungi was invariably accompanied by the development of a musty odor.

In the early stages of these investigations it was found that if the environment was suitable for the development of either cocci or spore-forming bacteria the fungi fail to multiply. Throughout the entire course of this study data conflicting with this have never been obtained.

Whether fungi or bacteria develop upon the cured leaf during fermentation is largely controlled by two factors; the composition of the leaf and the amount of moisture present. Data obtained to date do not indicate a definite critical moisture content below which fungi develop and above which bacteria inhibit their development that is applicable to all cigar-leaf tobacco. Rather it would appear that the amount of moisture necessary to the development of bacteria is definitely related to the composition of the tobacco or, in terms of the tobacco grower and processor, the quality of the leaf. In general, it has been found that leaf of good quality provides a suitable substrate for bacterial development at lower moisture content than does leaf of poor quality.

Cocci developing upon tobacco undergoing a satisfactory fermentation were found to be members of *Micrococcus* and *Staphylococcus*. Of these the *Micrococci* were usually present in far greater numbers. During later stages of the fermentation, coccoid forms were frequently found in greater numbers. These are thought to be identical with the very short rods described by Schmidt *(23)*. Biochemical studies of these forms have lead to the conclusion that they should be classified with the *Micrococci*.

Aerobic spore-formers associated with the satisfactory fermentation of the leaf have been identified as members of the *subtilis-mesentericus-vulgatus* group of *Bacillus*. These forms are undoubtedly the organisms described by Vernhout *(26)*, to the activities of which he attributed fermentation. They are also the type described by Schmidt *(23)* as associated with the satisfactory fermentation of Ohio tobacco of good quality. For the sake of brevity these will be referred to subsequently as *B. mesentericus*. *B. megatherium* and *B. mycoides* have been observed. These were described by Schmidt *(23)* on fermenting tobacco.

Rapid multiplication of cocci and bacilli upon fermenting tobacco was found to result in the establishment of anaerobic conditions in the center of the fermenting heap. Such conditions resulted in spoilage if allowed to continue. The facultative cocci and bacilli continued to multiply after the exhaustion of the oxygen and, in addition, *Clostrium* sp. became active. Putrid odors developed as the nitrogenous material was attacked anaerobically and eventually the cellulose was attacked and destroyed. Inasmuch as tobacco of good quality favored the most rapid growth of microorganisms, resulting in anaerobiosis in shorter periods than was true with tobacco of poorer quality, this condition was more frequently encountered in connection with the fermentation of the higher quality tobacco. Frequent aeration was found to prevent much of this loss.

Plate counts made during the progress of the fermentation showed that the rapidity of multiplication was more intimately related to moisture content and leaf composition than to other factors. Temperature of course proved of some significance, particularly in dealing with fermenting samples so small that the intense heat generated could not be retained. Temperatures of 35 to 40° C seemed well adapted to the development of those types associated with a satisfactory fermentation.

In dealing with leaf of at least fair quality held under optimum conditions a rise in plate count from less than 100,000 microörganisms per gram to counts of almost two billion per gram were ordinarily accomplished in less than three days. With the lower moisture content used in case fermentation, such rapid rise was not noted. If the tobacco, however, was undergoing any fermentative changes, a slow rise in plate count could be demonstrated.

The use of the direct count was found to provide much useful information in the study of fermenting tobacco. A rise in numbers during the course of the fermentation was always observed. Direct counts of cured tobacco, as previously stated, usually demonstrated the presence of 500 million to almost one billion spores and cocci per gram of tobacco. The number materially increased as fermentation progressed.

Of equal significance to the demonstration of the rise in numbers was the observation of chains of rods of members of *Bacillus*, conclusive proof of the multiplication of these organisms upon the tobacco. In dealing with spore-formers it is not always possible to be certain that an increase in count by the plate method actually implies multiplication. At times this merely reflects a change in the ability of spores to germinate when placed on the medium. The demonstration of great numbers of chains of the spore-former by the direct method, however, satisfactorily solved the problem as to whether the increased plate count implied reproduction on the leaf or increased germinability of spores already present on the tobacco at the beginning of the fermentation. No chains of spore-forming rods were observed on cured tobacco; apparently spore-formers are present on cured tobacco only in the spore state.

The investigations relative to the flora of fermenting tobacco conducted in this laboratory have shown that fungi, cocci and spore-forming bacteria are able to develop on cured tobacco. They have further shown that a substrate-moisture relationship exists which in large measure determines whether fungi will grow with consequent loss of tobacco through molding; or bacteria may develop with subsequent inhibition of the growth of fungi. These investigations have further demonstrated a substrate-moisture-oxygen relationship to exist which governs the loss of tobacco by rotting.

Relation of the microflora of cigar-leaf tobacco to catalase activity.—Having failed to confirm the findings of Loew *(14)*, in respect to multiplication of microörganisms upon fermenting tobacco, the authors attempted to determine whether a relationship existed between the enzymatic activity of the leaf and the microbial activity observed in this laboratory.

The enzyme catalase was discovered by Loew *(16)* on fermenting tobacco. The use of this enzyme in the present investigation does not imply the belief on the part of the authors that the enzyme is of any particular significance in the tobacco fermentation. The activity of catalase is easy to measure, however, and as it was considered of particular significance by Loew it was chosen for use in this work.

Table 1.—Effect of five-day storage at 37° C upon the microflora and catalase activity of cured tobacco adjusted to a moisture content of 30 per cent.

SAMPLE TAKEN	PLATE COUNT OF BACTERIA PER GRAM	ML. N/100 H_2O_2 DECOMPOSED BY .2-GRAM SAMPLE IN 60 MINUTES
Before storage	52,000	14.2
After 5 days storage	35,000,000	86.9

Several hundred determinations of catalase activity were made from the tobacco suspensions employed in the routine determinations of bacterial numbers on both cured and fermenting tobacco. A rather close correlation was found between catalase activity and microbial activity. In general any treatment of the tobacco attempted which resulted in inhibition of the bacterial population likewise prevented an increase in catalase activity. Conversely, treatments that gave an increase in bacterial numbers showed an increase in catalase activity. Table 1 shows the typical increase in numbers of bacteria and activity of catalase found in tobacco of average quality held for a few days under conditions suited to bacterial reproduction.

Table 2.—Effect of bacterial growth on the catalase activity of sterilized cured tobacco, adjusted to a moisture content of 35 per cent and held at 37° C.

SAMPLE TAKEN	INOCULATION	PLATE COUNT OF BACTERIA PER GRAM	ML. N/100 H_2O_2 DECOMPOSED BY .2-GRAM SAMPLE IN 60 MINUTES
Before storage	None	0	0
Before storage	Culture E-11*	31,000	0
After storage of five days	None	0	0
After storage of five days	Culture E-11	800,000,000	176.2

* Culture E-11 is an isolation made from fermenting tobacco identified with the *subtilis-mesentericus-vulgatus* group of *Bacillus.*

Heat treatments necessary to render tobacco sterile also rendered it free of catalase activity. It was found, as shown in table 2, that normal catalase activity could be imparted to sterilized tobacco by inoculation with organisms previously isolated from fermenting tobacco.

Table 3.—Changes in the microflora of Pennsylvania cigar-leaf tobacco of good quality undergoing a satisfactory case fermentation.

STAGE OF SAMPLING	DATE	PLATE COUNT OF BACTERIA PER GRAM	PLATE COUNT OF FUNGI PER GRAM	PREDOMINANT ORGANISM
At end of curing process	2/27/37	53,000	388,000	*B. megatherium*
After two months fermentation in case	4/26/37	1,840,000	11,000	*B. mesentericus*
After three and one-half months fermentation in case	6/14/37	1,160,000,000	None	Cocci

Johnson *(10)* reported that the sterilization of tobacco was accomplished by steaming for one hour, during which time the tobacco reached a temperature of 85° C. Such treatments in this laboratory failed to produce sterility, but intermittent exposure to live steam for 30-minute periods on three successive days proved satisfactory. Without employment of the intermittent method of sterilization, an exposure in the autoclave at 15 pounds pressure for a period of 150 minutes was found necessary to insure sterility.

Table 4.—Flora of cigar-leaf tobacco of inferior quality that showed unsatisfactory fermentation although cased for a period of four months.

SAMPLE	PLATE COUNT OF BACTERIA PER GRAM	PLATE COUNT OF FUNGI PER GRAM
Sample A	3,500	210,000
Sample B	90,000	45,000

A review of the data obtained in this laboratory leads to the conclusion that the measurements of enzymatic activity of the tobacco leaf made by Loew *(15, 16)* and other investigators of the tobacco fermentation were, after all, merely indirect measurements of microbial activity.

Microflora of case-fermented cigar-leaf tobacco.—Case fermentation of cigar-leaf tobacco as practiced by manufacturers has been studied in these investigations. Typical changes found in satisfactory case fermentation are represented in table 3. The slow rise in bacterial numbers as determined by plate count and the gradual decline in the viability of the fungus spores and mycelium present are characteristic of this practice. It is noted in table 3 that although aerobic spore-formers predominated on the platings at the end of the curing process and after two months fermentation, coccus forms outnumbered bacilli on the plates at the end of three and one-half months. Inasmuch as this tobacco was of the best quality and undergoing the normal fermentation, these findings are at variance with those of Schmidt *(23)*, who concluded that cocci were present in great numbers only on tobacco of inferior quality.

Table 5.—A study of the flora during the bulk fermentation of cigar-leaf tobacco which had previously undergone six years of slow fermentation.

SAMPLE	BULK TEMP. °F	PH	PER CENT MOISTURE	BACTERIAL COUNTS PER GRAM		PLATE COUNT OF FUNGI PER GRAM
				Plate *thousands*	Direct *millions*	
As received		6.6	12.8	70	900	1,400
Following water treatment		7.0	61.7	375,000	1,620	300
Following steaming		7.2	61.6	8,500	1,540	1,000
24 Hours after steam treatment		7.2	61.6	3.400	2,600	0
Following stemming		7.4	50.0	40,000	5,900	0
Bulked 36 hours	90	7.6	50.1	1,560,000	7,100	0
Bulked 72 hours	91	7.8	50.5	120,000	7,650	0
Bulked 5 days	92	above 7.8	50.4	312,000	7,100	0
Bulked 7 days	100	above 7.8	50.4	285.000	8.150	0
Bulked 9 days	112	above 7.8	51.3	64,000	7,900	0
Bulked 13 days	125	above 7.8	51.4	5,240	7,800	0
Bulked 17 days	131	above 7.8	51.6	4,350	8,200	0
Bulked 29 days	136 *rebulked*	above 7.8	51.3	520	8,450	0
Bulked 32 days	105	above 7.8	47.1	870,000	10,200	0
Bulked 49 days	136 *rebulked*	above 7.8	43.2	43,000	12,000	0
Bulked 52 days	110	above 7.8	43.0	230,000	14,500	0
Bulked 60 days	136 *rebulked*	above 7.8	42.7	400	14,700	0
Bulked 62 days	115	above 7.8	42.9	32,000	14,600	0
Bulked 69 days	136	above 7.8	40.1	1,130	15,000	0

Study was made of a number of tobaccos reported by the manufacturer as failing to ferment in the case. Table 4 presents the data obtained from two such samples that are typical of all received.

The counts presented in this table should be compared with those in table 3 representing determinations made at the end of three and one-half months of case fermentation. Table 3 shows the bacterial plate count on the normally fermenting tobacco to have reached a value in excess of one billion at this stage. On the other hand, table 4 shows that tobacco which has failed to undergo any case fermentation, although held for a period of several months in the case, gives a characteristic bacterial plate count of less than 100,000 organisms per gram. Fungi disappeared in the normally fermenting case at the end of three and one-half months but failed to do so in tobacco that had failed to initiate fermentation.

Microflora of bulk-fermented cigar-leaf tobacco.—Manufacturers of scrap chewing tobacco do not follow the same procedure in the fermentation of cigar-leaf tobacco as do cigar manufacturers. A common practice in the scrap chewing tobacco industry is to allow a slow fermentation of the leaf over a period of two to seven years, followed by a rapid fermentation at high moisture content in bulks containing several tons of tobacco. Bulk fermentation is completed in periods ranging from four to 10 weeks and the tobacco is then ready for manufacture.

Many bulks have been studied; data obtained from one that is characteristic of a satisfactory fermentation are presented in table 5. During the period of slow fermentation the moisture content of the tobacco had been allowed to fall below 15 per cent. At this stage the plate count of bacteria is ordinarily less than 100,000, the colonies on countable plates consisting for the most part of *B. megatherium.* It is significant that the mold count is ordinarily low at this time, much lower than the mold count of the freshly cured leaf.

The first operation in the bulk procedure is the moistening of the tobacco. Warm water is sprinkled on the baled tobacco as received; in this manner the contaminating material is largely washed away. The moistened bales are usually allowed to stand over night. During this time a significant rise in the bacterial count is always noted.

In addition *B. mesentericus* usually becomes the predominant organism at this stage and remains predominant throughout the bulk fermentation in many cases. Although some manufacturers stem the tobacco after the fermentation, the bulks followed in this study were stemmed before bulking. In this case the tobacco was steamed, stemmed and piled into bulks in the course of a few hours. The pH of the tobacco slowly rises during this period, table 5. Although the freshly cured tobacco examined in this laboratory was found to have pH values of 6 to 6.4, the tobacco following the slow fermentation was found to have pH values of 6.4 to 6.8.

Immediately after bulking there is a rapid rise in the bacterial plate count and likewise in the direct count. The latter reveals the presence of chains of spore-formers in the vegetative state. Cocci were found to predominate at this stage in the bulk from which the data for table 5 were obtained, and this was found to be the case in many bulks, although a number studied showed *B. mesentericus* to be predominant at this stage as well as throughout the remainder of the fermentation. The presence of both organisms in all bulks at all times was easily demonstrated.

The increase in bacteria was accompanied in every instance by the complete disappearance of viable fungi in the bulk and was accompanied by temperature rise and increasing alkalinity.

The bulks studied were turned and in that manner aerated when the temperature in the center of the bulk reached 136° F. This aeration cooled the tobacco to some extent and the cooler, outer leaves were always placed in the center of the new bulk. This aeration was always followed by another rise in the bacterial count which had decreased as the bulk became hot and oxygen supply deficient. Heating following the aeration proved possible as long as sufficient energy material remained to permit bacterial multiplication. From the data obtained in these investigations, it is concluded that the multiplication of certain bacteria on the leaf is intimately associated with the normal fermentation of cigar-leaf tobacco, and that the desired chemical changes are not obtained in the absence of such microbial activity.

SUMMARY

Cured tobacco of the cigar-leaf type has a characteristic microflora in which some selection has already taken place.

Selection continues during fermentation and favored types multiply profusely before the fermentation is completed.

Fungi, which are always present on the cured leaf, are destroyed rapidly in a satisfactory fermentation.

The multiplication of aerobic spore-forming bacteria and cocci characterize the normal fermentation.

Substrate-moisture-oxygen and temperature relationships determine the nature of the microbial activities and consequently the nature of the fermentation.

Unsatisfactory composition of the leaf, termed by the industry "poor quality," favors the multiplication of fungi. A moisture content insufficient for bacterial growth also favors multiplication of the fungi.

Consumption of oxygen by the bacterial population may lead to anaerobic conditions and subsequent spoilage due to anaerobic activities of microörganisms present.

Catalase activity of the leaf is closely related to the activities of the microörganisms present.

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9858

Effect of 1,2,5,6-Dibenzanthracene on *Fusarium lini.*

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In addition to being able to initiate malignant growth, the carcinogenic hydrocarbons have been shown to stimulate cell division of certain microörganisms. Since it has been postulated that cancer may be associated with an abnormal glucose metabolism, it was thought of interest to study the effect of a typical carcinogenic hydrocarbon on the growth and glucose utilization of *Fusarium lini.* This fungus has been shown to grow well on a medium composed of mineral salts and glucose as the only source of carbon producing a typical alcoholic fermentation.[1]

The carcinogenic hydrocarbon selected for this study was 1,2,5,6-dibenzanthracene. The culture medium had the following composition:

NH_4NO_3	1.00 gm.
$MgSO_4$	0.25
KH_2PO_4	0.50
Glucose	20.00

Distilled water to make 1,000 cc.

Since in preliminary tests the above hydrocarbon had no effect on the growth of *Fusarium lini* grown on the above medium, it was felt that this might be due to the low solubility of the hydrocarbon in the medium. Therefore in the work here reported a water-soluble oxidation product of the hydrocarbon was used. This was prepared by a method similar to that of Boyland and Boyland[2] as follows:

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[1] Anderson, A. K., *Minnesota Studies in Plant Science. Studies in the Biological Sciences,* 1924, No. 5, pp. 237-280.

[2] Boyland, E., and Boyland, M. E., *Biochem. J.*, 1934, **28**, 244.

A solution containing 0.123 gm. of 1,2,5,6-dibenzanthracene, dissolved in a small amount of anhydrous benzene, was exposed for 1¼ hours to the rays from a mercury arc ultraviolet light source. The solution was exposed directly to the light by being placed in a thin layer in one-half of a large Petri dish. From time to time benzene was added to replace that lost by evaporation. Finally the benzene was evaporated and the residue irradiated for 15 minutes. This residue was then extracted with several small portions of water, the washings filtered and the volume made up to 100 cc. This extract was used in the subsequent work.

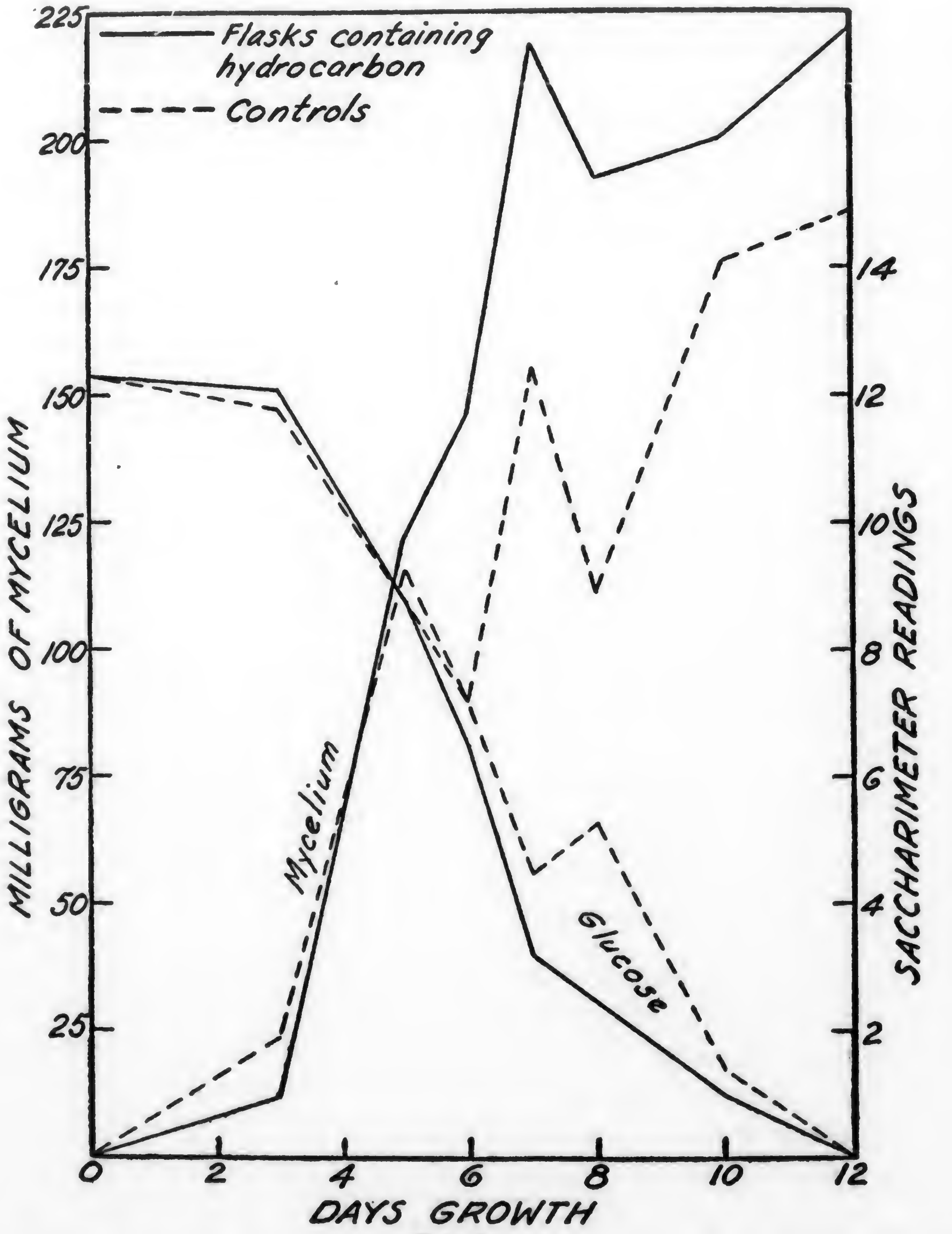

FIG. 1.

Showing mycelium production and glucose utilization of *Fusarium lini* as influenced by 1,2,5,6-dibenzanthracene.

A series of 16 Erlenmeyer flasks was prepared, each flask containing 100 cc. of medium. Eight of the flasks contained in addition one cc. of the water extract described above. After heat sterilization each flask was inoculated with a spore-mycelium suspension of *Fusarium lini*. The flasks remained at room temperature throughout the experiment. The flasks were arranged in pairs, with and without hydrocarbon. One pair was analyzed immediately for glucose by means of a saccharimeter. Sugar values are expressed as saccharimeter readings using a 400 mm. tube. At frequent intervals a pair of flasks was removed and the contents filtered through a Gooch crucible. A saccharimeter reading was taken on the filtrate, the mycelium being washed, dried and weighed. In all, three series were run. Similar results were obtained in all series. The results of one series are shown graphically in Fig. 1.

An examination of the data presented shows that water-soluble derivatives of 1,2,5,6-dibenzanthracene after an initial inhibiting period were effective in stimulating the activities of *Fusarium lini* as evidenced by an increase in the weight of mycelium and a more rapid utilization of glucose.

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ULLETIN

JUN

Studies on the
FERMENTATION OF TOBACCO

2. Microorganisms Isolated from Cigar-leaf Tobacc

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
STATE COLLEGE, PENNSYLVANIA

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PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION

BULLETIN 363* STATE COLLEGE, PA. JUNE 1938

Studies on the Fermentation of Tobacco

2. Microörganisms Isolated from Cigar-leaf Tobacco

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IN previous publications *(10, 11, 12, 13)* the authors have shown that the reproduction of certain microörganisms is associated with the successful fermentation of cigar-leaf tobacco and that the leaf exhibits a characteristic microflora, the predominating forms of which are determined by the substrate-moisture-oxygen-temperature relationships maintained during the course of fermentation.

The studies reported in this bulletin represent an attempt to describe morphologically and physiologically the microörganisms found in significant numbers on cured and fermenting cigar-leaf tobacco, which play an important part in the quality of the fermented product.

MATERIALS AND TECHNIQUE

The observations reported in this bulletin are based on the study of 2844 pure culture isolations of microörganisms from 354 samples of cured and fermenting cigar-leaf tobacco, table 1. The tobaccos from which these isolations were made were representative of the cigar-filler and binder types produced in Pennsylvania, Wisconsin and Ohio.

Isolations of cocci and aerobic spore-forming bacteria were made from dilution plates prepared in the routine examination of tobacco. In many cases isolations were made from a single colony of each colony type found on the plates at the time the count was made; in other cases isolations were made from all colonies appearing on the plates in the dilution counted. Although the first method furnishes a culture collection suitable for the identification of the various forms appearing during the course of the fermentation, the second method is essential to a study of the physiological adaptations taking place as the fermentation proceeds, and observations of such a nature are based on this second method of collecting cultures.

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*Authorized for publication April 5, 1938.

Table 1.—Pure culture isolations from different samples of cigar-leaf tobacco.

ISOLATED FROM	NUMBER OF SAMPLES	NUMBER OF ISOLATIONS OF				TOTAL
		Cocci	Aerobic Spore-formers	Other Bacteria	Fungi	
Cured Pennsylvania, 1937 crop	15	7	109	17	210	343
Cured Pennsylvania, 1936 crop	112	5	419	3	317	744
Cured Wisconsin, 1937 crop	9	3	57	2	141	203
Case fermenting Pennsylvania, 1936 crop	22	124	71		35	230
Bulk fermenting Pennsylvania, 1930 crop	41	56	145	1		202
Laboratory fermenting Pennsylvania, 1936 crop	76	59	233	2	7	301
Bulk fermenting Wisconsin, 1931 crop	36	61	139	5	43	248
Laboratory fermenting, Wisconsin 1937 crop	31	385	42	22	19	468
Bulk fermenting Ohio, 1932 crop	10	11	40		3	54
Case fermenting Maryland, 1936 crop	2		4		47	51
Totals	354	711	1259	52	822	2844

Crystal violet in an appropriate concentration, usually 1:200,000 was added to the nutrient agar used in plates made for the purpose of determining the presence of gram-negative bacteria. All bacterial colonies appearing on these plates were examined microscopically and isolations were made from all colonies found to be gram-negative as well as from colonies the Gram reaction of which at this time was not well defined. In further efforts to determine the presence of gram-negative bacteria, crystal violet was added to beef-extract peptone glucose agar. Dilution plates were poured with this medium and colonies picked as previously described.

Isolations of anaerobes were made from deep tubes of crystal violet beef-extract peptone glucose agar. With a concentration of gentian violet of 1:200,000 the colonies of *Micrococcus* and *Bacillus* ordinarily far outnumbered the colonies of the genus *Clostridium* in this substrate, rendering isolation of the anaerobes somewhat difficult. The pyrogallol method of obtaining anaerobic conditions in the tubes was used in some instances.

Cellulose-fermenting species of *Clostridium* were isolated by the enrichment method, using the medium of Omeliansky and incubating duplicate tubes at 37 and 55° C.

Morphological identifications of fungi were made from the growth on glucose-peptone-acid agar. Isolations were made from colonies not easily identified in this manner and were the subject of further study.

Cultural studies useful in the classification of the several bacterial forms found were made in all cases. These studies included all morphological and physiological information found to be of value in comparing these isolations with species and other groupings described by Bergey *(2)*, Lehman and Neuman *(9)*, Hucker *(5)*, and Smith and Clark *(16)*.

Micrococcus isolations that showed a tendency to appear in grapelike clusters were investigated serologically according to the method of Julianelle *(7)*.

MICROCOCCI OF CIGAR-LEAF TOBACCO

The presence of gram-positive cocci upon most samples of cured and fermenting cigar-leaf tobacco is easily demonstrated. Although greatly outnumbered by the spore-formers on the cured leaf, the cocci have been found to multiply rapidly during the early stages of active fermentation, frequently reaching a number by plate count, in excess of 1,000,000,000 cells per gram. As a rule their numbers decline rapidly following this maximum and it becomes increasingly difficult to prove the presence of this coccus form in the later stages of the process.

Morphological classification.—The cocci found upon cigar-leaf tobacco may be roughly classified upon the basis of morphology, in three types:

TYPE I.—Cells small, occurring in grapelike clusters.

TYPE II.—Cells somewhat larger than in Type I and occurring singly, in pairs, and in small clusters.

TYPE III.—Cells much larger than in either of the preceding types, somewhat oval, occurring singly, in pairs and in clusters. Such a classification, however, is abritrary and extremely unsatisfactory, inasmuch as more than half the isolations occupy intermediate positions with respect to the types described. In fact, the culture collection of 711 cocci isolated from tobacco ranges from the extreme of Type I to the extreme of Type III with no clear-cut lines of demarcation separating the types. Type I has ordinarily been found to be associated with the earlier stages of the fermentation, Type II with that period in which the rise in numbers is most rapid and Type III with the period of highest bacterial count.

Classification upon the basis of pigment production reveals that almost all isolations from tobacco undergoing a satisfactory fermentation are non-pigmented forms. A few pigment formers have been isolated from cured tobacco and from tobacco held at a moisture content too low for active fermentation.

Gelatin liquefaction and activity in litmus milk offer a basis for the classification of these organisms which might appear satisfactory. A cultural description of the five types and one heterogeneous group differentiated in this manner with the number of isolations falling into the various types is given in table 2.

Table 2.—Classification of 711 cultures of micrococci isolated from cured and fermenting tobacco, upon gelatin liquefaction and action in litmus milk. 14 day incubation.

ACTION UPON	TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	GROUP VI
Gelatin	Crateriform	Crateriform	Crateriform	No liquefaction	No liquefaction	Rapid, other than Crateriform
Litmus milk	Acid curd	Acid, no curd	No change	Acid, no curd	No change	Proteolysis
Dextrose	+	+	+	+	−	±
Sucrose	+	+	+	+	−	±
Lactose	+	+	±	+	−	±
Mannitol	+	+	+	−	−	−
Growth at 55° C	−	−	−	−	+	−
Number of isolations	189	151	73	51	204	43

In this biochemical separation, as in morphological separation, classification rests upon purely arbitrary decisions. An incubation period of two weeks was the basis of the separation upon gelatin and litmus milk reactions. Any other incubation period would give different groupings of the cultures; shorter incubation would move many of Type I into Type II, many of Type II into Type III, etc. On the other hand a longer incubation woud move many of the Type IV and V cultures into Types I, II or III, some Type III cultures into Type II and some Type II cultures into Type I. In other words, cultures classified in one type often differ from those in another type not in the inherent ability to carry out a certain reaction but in the speed with which the particular cultures are able to bring it about.

Inasmuch as attempts to classify cultures of micrococci obtained from tobacco suggested the possibility of physiological adaptations to an ever-changing environment, a series of 217 isolations of micrococci from a single small experimental bulk of fermenting tobacco were made in the course of 18 days. Dilution plates were poured with sufficient interpolations in the series to obtain each day a plate with from 20 to 40 colonies. Isolations were made from all colonies appearing on these plates and a study was made of the morphological and physiological characteristics of the cultures of cocci.

The morphological classification of the daily isolations of cocci are shown in figure 1. Although the isolations made the first day of the fermentation were easily classified as staphylococcus-like in appearance, and most of those isolated toward the end of the 18-day fermentation were obviously large oval-celled cultures, many if not most of the cultures isolated from the third to ninth days were classified arbitrarily as these isolations represented morphological intermediates. The two extremes are shown in figures 2 and 3.

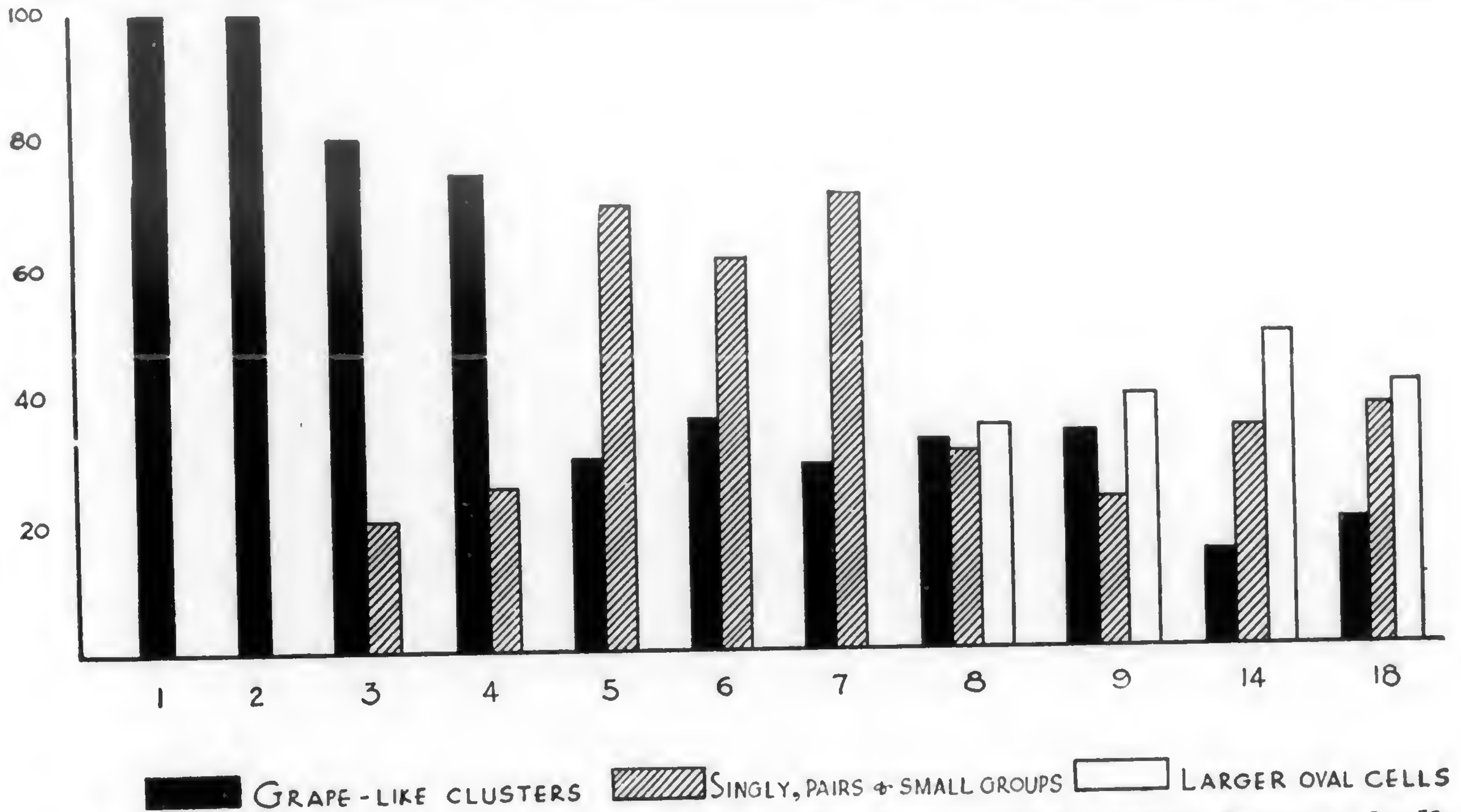
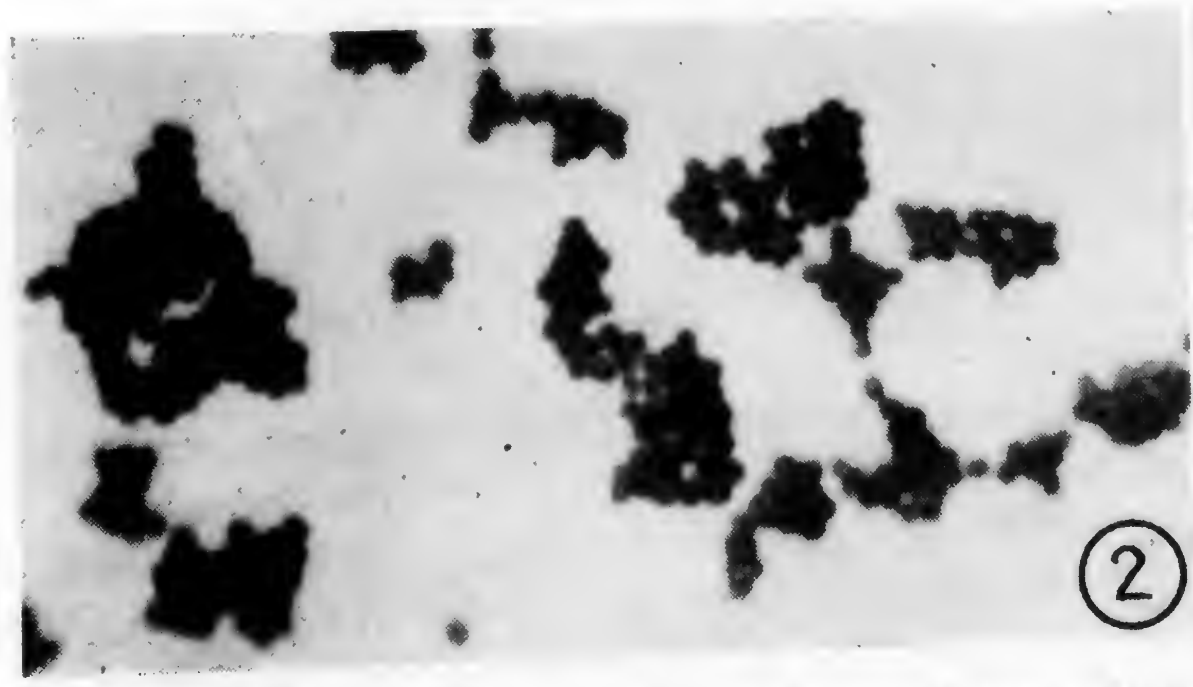


Fig. 1.—Morphological classification of micrococci isolated from a bulk of fermenting tobacco.



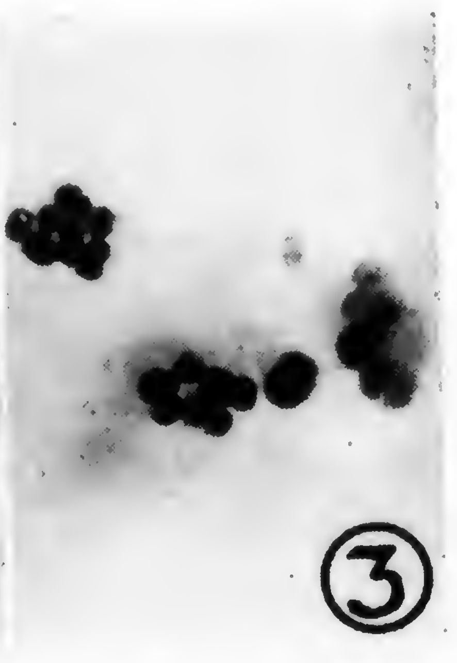


Fig. 2.—Micrococci isolated in the initial stage of cigar-leaf fermentation. The grape-like clusters are characteristic of the cocci at this stage. (950 X)

Fig. 3.—Micrococci isolated at the most active stage of fermentation. The large oval cells are characteristic of the cocci at this stage. (950 X)

Figure 1 emphasizes the relation of morphological type to the stage of the fermentation, confirming previous conclusions made on the basis of more or less random isolations. A study of the 217 cultures reveals that the change is indeed a gradual one.

Classification by gelatin liquefaction and action in litmus milk.—The description of the five types and one heterogenous group listed in table 3 is presented in table 2. Table 3 reveals a daily change in the biochemical activities of the predominant cocci of the bulk.

Table 3.—Cultural classification of 217 cultures of micrococci isolated from a bulk of fermenting tobacco during a period of 18 days.

DAY OF ISOLATION	TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	GROUP VI
First	27	3	0	0	0	0
Second	14	6	0	0	0	0
Third	19	4	1	0	0	0
Fourth	3	4	0	0	0	0
Fifth	5	3	3	0	0	0
Sixth	6	3	26	3	0	0
Seventh	3	2	2	0	0	0
Eighth	0	8	7	4	1	0
Ninth	0	6	0	2	10	3
Fourteenth	0	1	0	2	10	5
Seventeenth	0	4	0	1	15	0
Total cultures	77	45	39	12	36	8

Starting with an active type of organism capable of liquefying gelatin, producing an acid curdling of milk and fermenting several of the common sugars, the change proceeds by degrees to a form incapable of liquefying gelatin, producing no change in milk and unable to ferment the common carbohydrates. Gelatin liquefaction of these organisms is shown in figure 4.

Figure 1 and table 3 show that there is some relation between morphology and cultural characteristics. Micrococci occurring in grape-like clusters usually fall in Types I or II of table 2, and the larger oval-celled forms more often fall in the relatively inert Type V. But the relationship is not an exact one and morphological types are scattered throughout the six biochemical groupings.

The speed of the reaction rather than the reaction itself determines, in many cases, the classification made in tables 2 and 3. The speed of these reactions is of interest in this connection, particularly with reference to gelatin liquefaction and changes in litmus milk.

That the changes in the ability to liquefy gelatin as the fermentation of the bulk proceeded are rather gradual in nature is shown in table 4. All gelatin liquefiers among the 217 isolations made from this experimental bulk were included in table 4 with the exception of the eight cultures classified in Group VI, table 3. These cultures liquefied the gelatin rapidly, in either saccate or stratiform manner and apparently bear little relation to the other 209 isolations from the bulk.

All micrococcus isolations from the bulk have been cultured in litmus milk and gelatin several times and the results have agreed extremely well. The cultures have not been held within the laboratory for sufficient time to make any statement as to retention of initial characteristics on prolonged laboratory cultivation.

Table 4.—The speed of gelatin liquefaction by micrococci isolated during the course of 18 days from a fermenting bulk of cigar-leaf tobacco.

DAY OF ISOLATION	PER CENT OF COCCI GIVING CRATERIFORM GELATIN LIQUEFACTION	DAYS OF INCUBATION AT 20°C NECESSARY TO OBTAIN EVIDENCE OF LIQUEFACTION
First	100	1– 2 days
Second	100	2 days
Third	100	3 days
Fourth	100	3– 4 days
Fifth	100	4 days
Sixth	90	4– 5 days
Seventh	80	5 days
Eight	80	5– 6 days
Ninth	50	10 days
Fourteenth	38	10 days
Seventeenth	20	10–12 days

The litmus milk reactions of this group of cultures present a picture strikingly similar to that presented by the gelatin characteristics. As daily isolations were made there was first noted a gradual change in the length of time required to coagulate milk, followed by an increasingly larger percentage of isolations incapable of coagulation. This, in turn, was accompanied and followed by changes in the ability to produce an acid reaction in milk, the length of time required for such acid production becoming increasingly longer. Eventually most isolations were unable to produce changes in milk during the 14-day period of incubation. With regard to the utilization of carbohydrates and alcohols as sources of energy, the loss of the ability to utilize mannitol was first noted, followed in turn by the degree of acidity produced in lactose, the length of time required to produce acidity in any of the carbohydrate media employed and finally the loss of the ability to ferment the common carbohydrates.

Two theories may be advanced to account for the changing morphological and physiological characteristics of the isolations of micrococci from tobacco undergoing fermentation. It may be assumed that the fermentation is characterized by a succession of species or physiological types of cocci, one species replacing another in rapid succession; or that the isolations merely reflect physiological adaptations of a species to a constantly changing environment. Perhaps some of the changes noted may actually represent succession of species and others physiological adaptation.

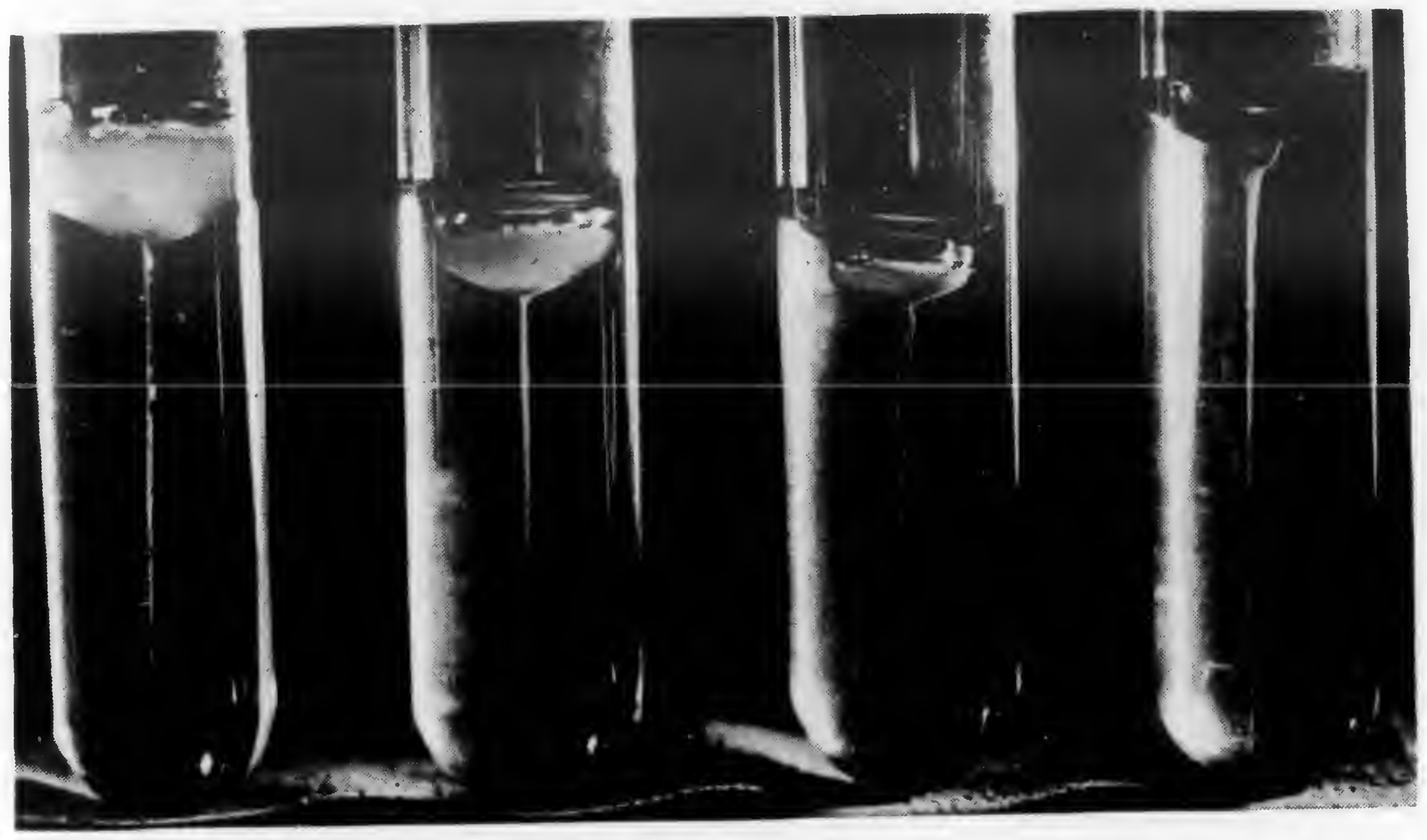


Fig. 4.—Gelatin tubes inoculated with micrococci isolated at successive stages in the fermentation. Incubation 14 days. Tube at extreme left inoculated with isolation obtained on the first day of fermentation; tube on the extreme right inoculated with isolation made the fourteenth day of the fermentation. Note loss in speed with which liquefaction of gelatin takes place.

In further efforts at classification other cultural characteristics of the 209 isolations listed as Types I to V inclusive in table 3 were determined. All proved able to reduce nitrates to nitrites, none was able to grow on the mono-ammonium phosphate medium of Hucker *(5)*, none was able to utilize urea and growth on potato was slow, restricted and yellow to white in color. The precipitin test of Julianelle *(7)* showed no relation between these isolations and either the A or B groups of staphylococci.

Although it is not suggested that this collection of cultures represents species hitherto undescribed, nevertheless the schemes of classification of Hucker *(5)* and of Bergey *(2)* offer little help in the classification of the various types of this collection. The scheme of Lehmann and Neumann *(9)* is more useful. Types I to IV, table 3, might be considered as varieties of *Micrococcus candicans* as described by Lehmann and Neumann *(9)*. Type V might be considered as closely related to *Micrococcus viticulosus* of the same

authors. There is much more latitude in the definition of a species by Lehmann and Neumann *(9)* than by either Hucker *(5)* or Bergey *(2)*. However, in the consideration of such a tentative classification, the morphological characteristics of *Micrococcus viticulosus,* although corresponding with the characteristics of a majority of the forms classified in table 3, Type V, are found to a lesser extent in most of the other types of table 3.

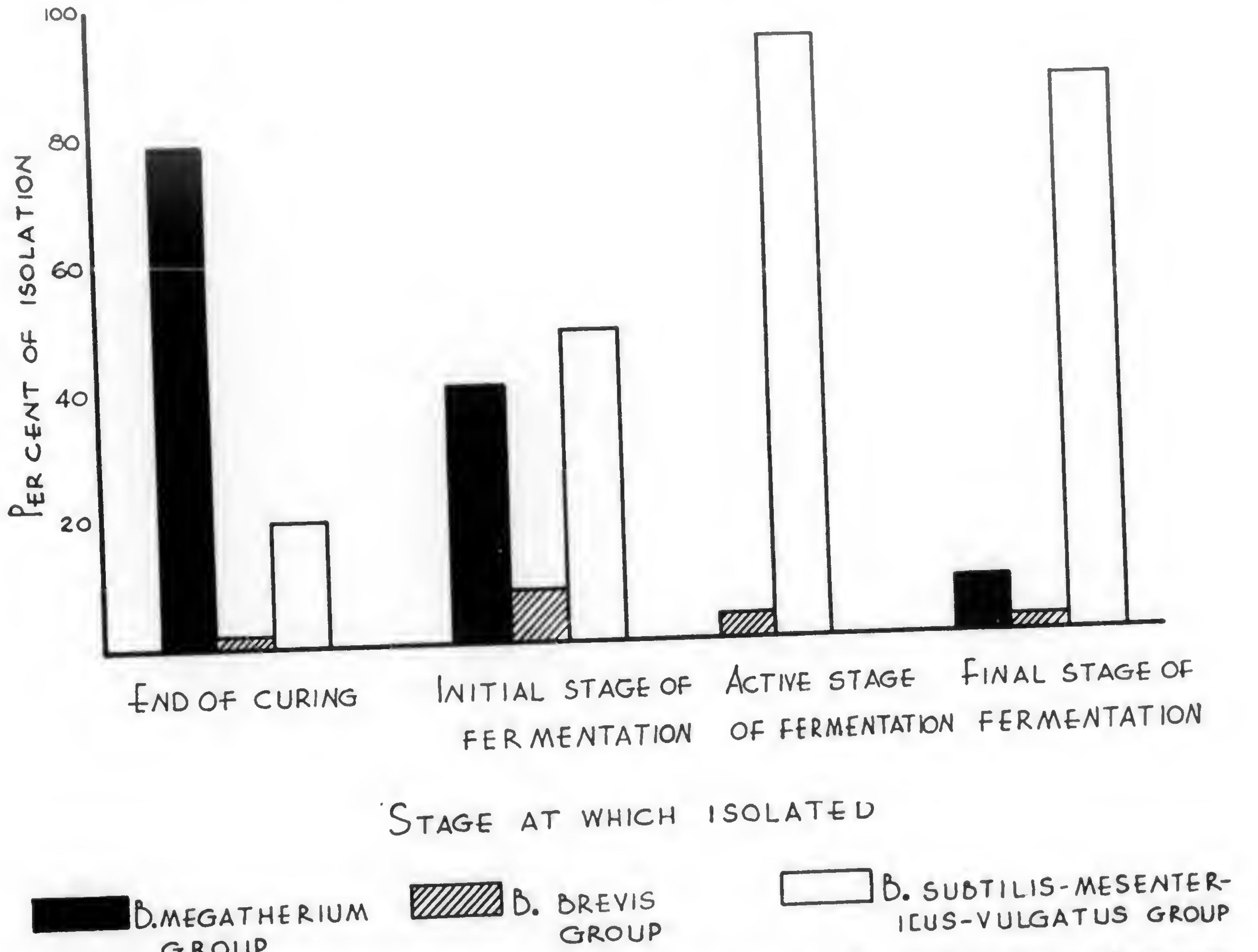


Fig 5.—Relative numbers of the three groups of aerobic spore-formers found on cigar-leaf tobacco at various stages in the processing.

The presence of micrococci on fermenting tobacco has been noted by Schloesing *(14)* and Suchsland *(17)*. Koning *(8)* described an organism that he termed *"Diplococcus tabaci"* found on fermenting tobacco. This was described, however, as gram-negative. Schmidt *(15)* observed cocci on tobacco of poor quality. Johnson *(6)* found staphylococci on fermenting leaves. Giovannozzi *(4)* found cocci on the fermenting product and reports that Rossi and Volganow also found this form associated with the fermentation. Giovannozzi's description of the cocci found by Rossi is of particular interest. Termed *Micrococcus nicotianae,* the organism was oval, .9 x 1.2 micra, gram-positive, facultative, producing no change in milk and failing to ferment sucrose and to liquefy gelatin. The organism described by Rossi as reported by Giovannozzi *(4)* bears a striking similarity to Type V, table 3.

Occurring frequently in numbers exceeding 1,000,000,000 per gram, the importance of micrococci in the fermentation of cigar-leaf tobacco cannot be denied. Inasmuch as these have been found upon tobacco of the highest quality as well as upon inferior grades and in fermentations pronounced entirely satisfactory by the manufacturer, micrococci must be considered important members of the normal flora of the fermenting cigar-leaf.

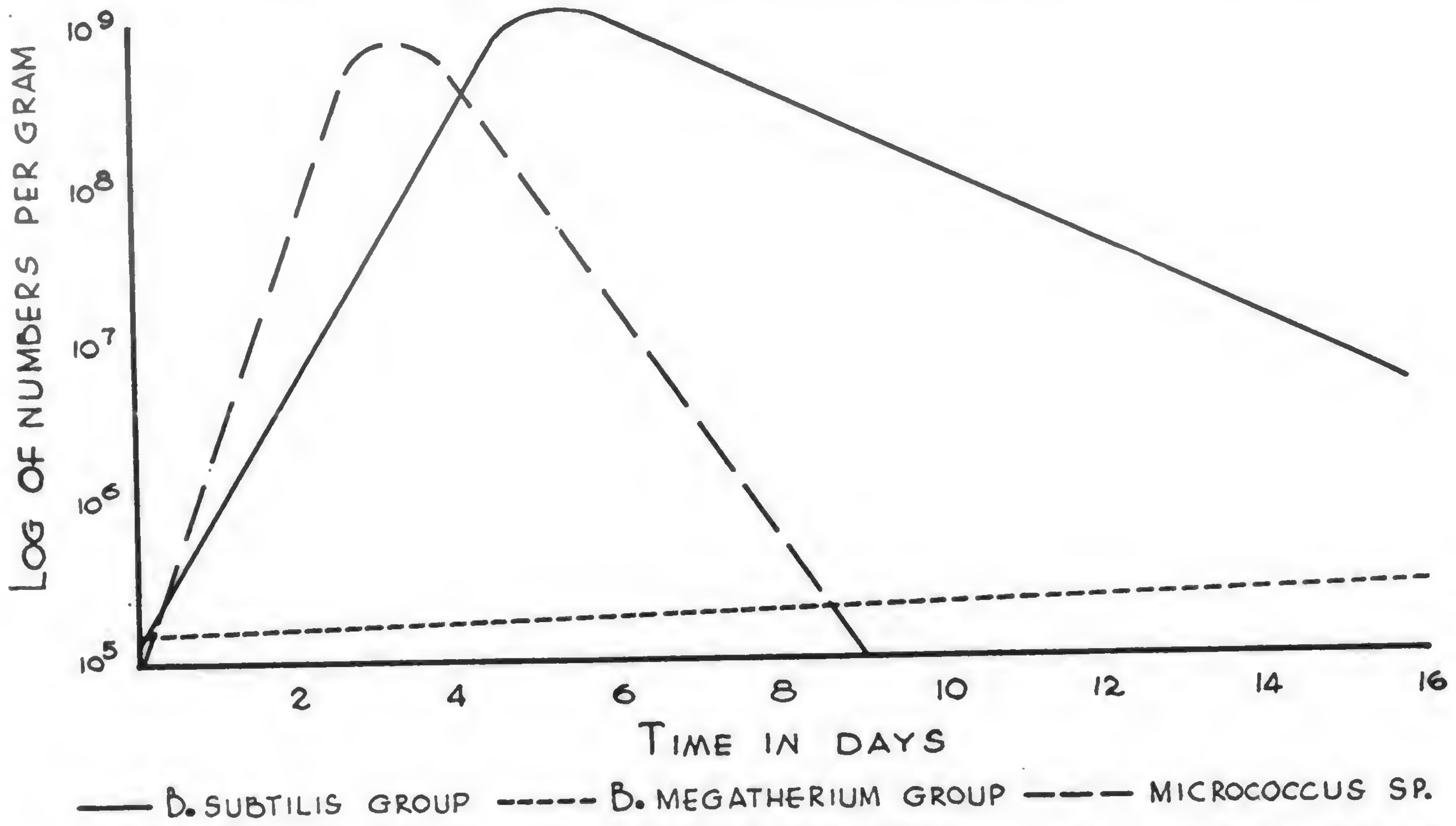


Fig. 6.—Prepared from data obtained in a laboratory fermentation, showing the numbers of the *B. subtilis* group, *B. megatherium* group and cocci found throughout the course of 16 days. In a general way this corresponds with data obtained from commercial fermentation of the rapid type.

BACILLI OF CIGAR-LEAF TOBACCO

All samples of cigar-leaf tobacco examined in this laboratory have shown the presence of aerobic spore-formers. The numbers found have varied from a few thousand per gram, in some samples of cured tobacco and improperly fermenting tobacco, to counts in excess of 1,000,000,000 per gram in samples taken from the active stages of fermentation.

More than 1200 pure culture isolations of this group of bacteria were studied. The scheme of Smith and Clark *(16)* has been found useful in an attempt to classify these isolations. Classified according to this scheme the isolations have fallen into three groups, the *Bacillus megatherium–cereus* group, the *B. subtilis–mesentericus–vulgatus* group and the *B. brevis* group, table 5, and figure 5.

Organisms of the *B. megatherium–cereus* group liquefy gelatin rapidly, produce no gas in carbohydrate media, store reserve materials which may easily be demonstrated in the cell, lose their gram-positive characteristics within a short time following transfer, produce a rather large cell containing central to eccentric spores, frequently are found in rather long chains, grow best in the temperature range of 25 to 35° C and fail to grow at a temperature of 50° C.

Table 5.—Classification of the aerobic spore-forming bacteria isolated from cigar-leaf tobacco.

ORGANISM		STAGE AT WHICH ISOLATED				TOTAL
		End of Curing	Fermentation			
			initial stage	*active stage*	*final stage*	
B. megatherium group		463	57	0	21	541
B. brevis group		9	12	11	5	37
B. subtilis–mesentericus–vulgatus group	Type A	21	10	65	42	138
	Type B	15	16	30	22	83
	Type C	37	21	155	16	229
	Type D	40	23	35	133	231
Total		585	139	296	239	1259

A microphotograph of a member of this group is presented in figure 7. This is the characteristic bacterial group of cured tobacco, usually outnumbering all other bacterial groups on plates inoculated from the cured leaf. It has not been found to reproduce to any great extent during the normal fermentation, although it may often be found in slightly greater numbers at the end of the fermentation than at the beginning. More than one species of this group is found, as a rule, in the plating of cured tobacco. *B. megatherium* and *B. cereus* are most often encountered although *B. mycoides* and other members of the group are occasionally noted in the plating of cured tobacco. As shown in table 5, members of this group have not been found on plates made during the active stages of fermentation, too many members of *Micrococcus* and the *B. subtilis–mesentericus–vulgatus* group being present at that stage for the appearance of the *B. megatherium–cereus* group on countable plates. On the basis of this study it is concluded that any rôle the *B. megatherium* group may play in the fermentation is a minor one.

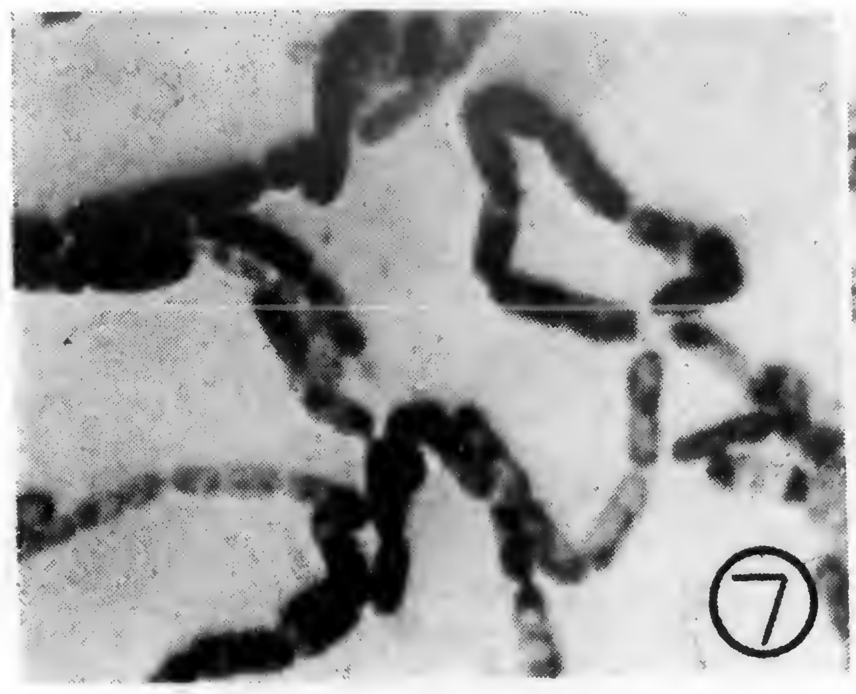
Organisms of the *B. subtilis–mesentericus–vulgatus* group liquefy gelatin, do not produce gas from carbohydrates, do not store reserve fat, produce acetyl-methyl-carbinol, usually retain gram-positive character much longer than do members of *B. megatherium–cereus* group, produce a rather small cell as contrasted with many members of the genus, spores are ordinarily central to eccentric, cells often found singly, in pairs or short chains, and grow over a rather wide range of temperature, many forms growing at 55° C. This is the characteristic spore-forming group of fermenting tobacco. During the course of the actual fermentation of the tobacco this group has never been found to be outnumbered by any form other than micrococci and then for only a limited period.

Table 6.—Distinguishing cultural characteristics of the four types of the *B. subtilis-mesentericus-vulgatus* **group found on fermenting cigar-leaf tobacco.**

	TYPE			
	A	B	C	D
Growth on potato	Very slow, pink, glistening, vesicular	Rapid growth, pink, smooth	Dark gray to brown, smooth or rough	Wrinkled, without pigment or pink
Growth on ferric ammonium citrate tryptone agar	Pink, vesicular, spreading	Rough, dry, netted, cream colored		
Liquefaction of gelatin	Crateriform becoming stratiform	Saccate	Saccate	Saccate
Litmus milk	Coagulated without acid, serum zone, slow proteolytic action			Rapid complete digestion
Galactose	Ferments with pellicle	Variable	Variable	Does not ferment
Raffinose	Does not ferment	Ferments	Does not ferment	
Growth at 55° C	Good	Variable		None

All isolations of this group from tobacco grow well on a synthetic substrate utilizing an ammonium salt as the source of nitrogen. Energy is readily supplied by the citrate and malate ions and by a number of the common carbohydrates. Cultivation upon laboratory media shows that the isolations belonging to this group may be roughly classified into four types. The incidence of these four types, termed A, B, C and D for convenience, is shown in table 5 and the distinguishing characteristics in table 6.

Type A of the *B. subtilis–mesentericus–vulgatus* group is characterized by ability to grow well at 55° C, formation of a pellicle on many substrates in which the other three types of this group fail to form pellicles, peculiar pink vesicular growth on ferric ammonium citrate tryptone agar, and crateriform to stratiform liquefaction of gelatin. The colony formation of this organism when cultivated at 45° C or lower is peculiar, the colony appearing as a rosette covered with vesicles. Higher temperatures frequently result in a slimy type of growth, a phenomenon common with this type of organism. Type A has been found on all satisfactorily fermenting cigar-leaf tobacco examined and is present in great numbers during the active stage of the fermentation. Without doubt this type is of significance in the fermentation process. It is probably a variant of *B. subtilis*.



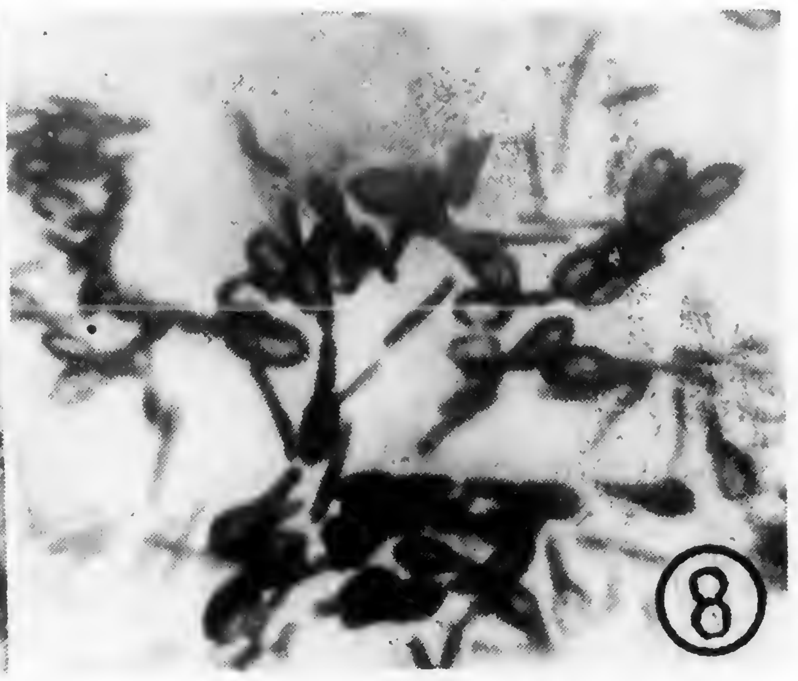


Fig. 7.—Microphotograph of a typical member of the *Bacillus megatherium–cereus* group. (950 X)

Fig. 8.—Microphotograph of a typical member of the genus *Clostridium* isolated from an early stage of bulk rot. (950 X)

Type B has also been found on most fermenting tobacco examined but usually in smaller numbers than Type A. This form differs from the other three types in ability to ferment raffinose and growth on potato, which is rapid, smooth and pink in color. Activity in litmus milk is similar to that of A and C; liquefaction of gelatin and growth on ferric ammonium citrate similar to that of C and D. Although this type bears a certain relation to *B. subtilis* in many respects it more closely resembles *B. vulgatus* and might be considered a variant of the latter. Gelatin liquefaction of these organisms is shown in figure 9.

Type C bears a certain resemblance to *B. subtilis* but in some respects more closely approximates *B. mesentericus*. This form grows well at 45° C while growth at 55° C is variable. It is characterized in particular by growth on potato which is similar to that of *B. mesentericus*. It has been found on all cigar-leaf tobacco examined and is usually predominant during the period of active fermentation. It is seldom found in the late stages of fermentation. Type D is the predominant type during the final stages of the fermentation of cigar-leaf tobacco from Ohio and Wisconsin. More than half of all isolations made at that phase have been of this type when the tobacco was produced in Ohio or Wisconsin. This type has not been isolated from Pennsylvania crops. It differs from the other three types in its activity in milk, completely digesting the milk without coagulation within a period of 48 hours. The character of the growth on potato also differs from the other types. The temperature range of this type is not as wide as in the case of the other three. It grows best at 37° C, very little at 45° C and fails to grow at 55° C. It is probably a variant of *B. vulgatus*.

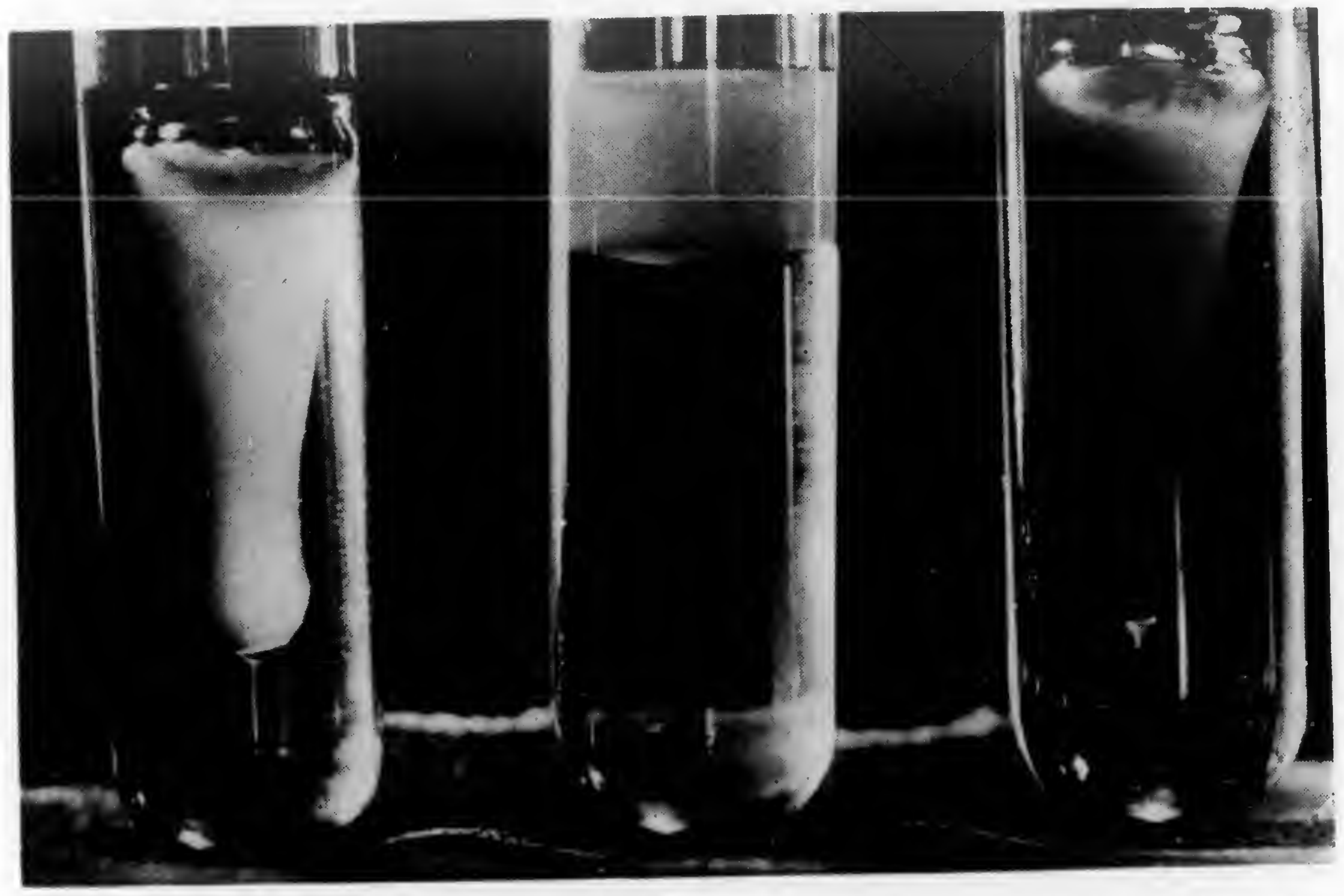


Fig. 9.—Gelatin liquefaction by aerobic spore-formers isolated from tobacco. From left to right, liquefaction typical of the *B. megatherium* group, Type A of the *B. subtilis–mesentericus–vulgatus* group, Type B of the same group. Types C and D react as does B.

That these four types are distinct is open to question. The characteristics exhibited at the time of isolation have been retained in the laboratory for more than a year. Type A differs sufficiently from the others to be set apart. The other three might be combined in one or two types.

From the data collected in this laboratory the conclusion must be drawn that the significance of the *B. subtilis–mesentericus–vulgatus* group in the fermentation of cigar-leaf tobacco is great. Reproduction to the extent noted in this group cannot be accomplished without important modification of the substrate.

The *B. brevis* group has been encountered less often than either of the other two groups of aerobic spore-formers. It has not been found on fermenting Pennsylvania tobacco although isolated from tobacco grown in Ohio and Wisconsin. It is apparently never present in great numbers when compared with the numbers of the *B. subtilis–mesentericus–vulgatus* group. Reproduction must take place to some extent during the fermentation, however, as it has been isolated a number of times from the high dilutions plated during periods of active fermentation. This group differs from the *B. subtilis–mesentericus–vulgatus* group in inability to produce acetyl-methyl carbinol, very slow proteolysis of milk and inability to hydrolyze starch.

Schloesing *(14)* and Suchsland *(17)* described organisms on fermenting tobacco which were probably aerobic spore-formers. Davalos *(3)* found bacilli and described five types, some of which were undoubtedly identified with the *Bacillus subtilis–mesentericus–vulgatus* group. Behrens *(1)* specifically mentioned *B. subtilis* and Vernhout *(18)* definitely identified the tobacco organisms studied as members of the *B. subtilis* group. Koning *(8)* reported both gram-positive and gram-negative organisms and among the former described some of his types as related to *B. subtilis* and *B. vulgatus*. Schmidt *(15)* reported *B. mesentericus* on Ohio tobacco of good quality. Johnson *(6)* observed "large bacteria" and showed colony pictures which leave little doubt that members of the genus *Bacillus* were encountered by him. Giovannozzi *(4)* stated that *B. mesentericus, B. subtilis, B. megatherium* and *B. mycoides* were found by Rossi on fermenting tobacco.

It is concluded from the data presented in this and the preceding section that members of the genera *Bacillus* and *Micrococcus* are the microörganisms definitely associated with the fermentation of cigar-leaf tobacco. Figure 6 shows the numbers of these two genera present in a typical fermentation.

OTHER MICROÖRGANISMS

The presence of large numbers of aerobic spore-forming bacteria and cocci renders the use of a bacteriostatic agent necessary in any attempt to determine the presence of gram-negative bacteria upon tobacco of the cigar-leaf type. For this purpose crystal violet was chosen and various concentrations utilized in an effort to inhibit the gram-positive forms and permit the growth of the gram-negative bacteria.

From the cured leaf, particularly if the leaf is diseased, it is not difficult to isolate gram-negative rods of the type *Achromobacter* and *Flavobacterium*. Doubtless many of these gram-negative forms are members of the genus *Phytomonas* but the attempt has not been made to identify these isolations as such.

If fermentation progresses in a satisfactory manner it becomes very difficult to demonstrate the presence of gram-negative forms. The few colonies developing upon crystal violet agar are, as a rule, members of *Micrococcus* and *Bacillus* groups which the dye has failed to inhibit. Gram-negative bacteria cannot be assigned any significant rôle in the fermentation of cigar-leaf tobacco, on the basis of data accumulated in this laboratory. A few investigators, such as Koning *(8)*, have reported gram-negative organisms, but it is not clear that the type of tobacco examined was comparable to the cigar-leaf of Pennsylvania, Ohio and Wisconsin. That gram-negative forms multiply upon certain of the cigarette types is easily demonstrated.

The presence of members of the genus *Clostridium* can be shown by anaerobic incubation using crystal violet in the substrate to inhibit the facultative cocci and bacilli. These forms are either undergoing some multiplication in the normal fermentation or the spores are becoming more subject to germination on laboratory media as the fermentation progresses, as it becomes less difficult to demonstrate this type in the later stages of the fermentation. In incipient spoilage due to lack of aeration, and in actual rotting of the tobacco, these forms are found in large numbers. A microphotograph of a member of this genus isolated from tobacco is shown in figure 8. Oval, terminal spores seem characteristic of *Clostridium* found on tobacco.

Examination of tobacco in the early stages of rotting in the bulk fermentation reveals the presence of *Clostridium* of the cellulose-fermenting type. Isolation of these is accomplished in the medium of Omeliansky, pure cultures of types multiplying at 55° C having been obtained in this manner, following several enrichment transfers. Growth at 37° C of a similar type has been shown, but this has been found less frequently than has the thermophilic form.

Fungi on cured cigar-leaf tobacco.—The presence of fungi in large numbers is characteristic of cured cigar-leaf tobacco. More than 70 per cent of the fungi forming colonies on the laboratory media have been identified as members of the genera *Penicillium* and *Aspergillus*, the former genus being found more often than the latter. The several other colonies examined proved to be representative of a number of genera of the type commonly encountered in the examination of soil and plant material. Inasmuch as all these forms disappeared within a short time in the normal fermentation, it is thought that they have no important rôle in the process. Torula have occasionally been encountered on acid agar plates inoculated from fermenting tobacco, but never in significant numbers.

SUMMARY

Microörganisms of cured and fermenting cigar-leaf tobacco that were found in significant numbers have been isolated and studied in pure culture.

The predominant forms upon the cured leaf were bacteria of the *Bacillus megatherium* group and fungi of the genera *Penicillium* and *Aspergillus*.

Satisfactory fermentation is associated with a rapid rise in numbers of bacteria of the *Micrococcus candicans* type and the *Bacillus subtilis–mesentericus–vulgatus* group.

The predominant types upon the cured leaf apparently play little if any part in a satisfactory fermentation, viable fungi disappearing entirely in the early stages of the fermentation and bacteria of the *Bacillus megatherium* group failing to show any significant increase in number during the process.

The morphological characteristics of the cocci multiplying upon cigar-leaf tobacco, range from grapelike clusters of small spherical cells in early stages to large, oval-celled forms in later stages.

Many of these cocci resemble *Micrococcus candicans* in cultural characteristics. This type, however, is preceded by a somewhat more active type (physiological activity) and followed by a less active form.

The cultural characteristics of the bacteria of the *B. subtilis–mesentericus–vulgatus* group reproducing on fermenting tobacco allow division into four types. Three of these types are found in great numbers during the more active stages of the fermentation. The fourth is usually found in the final stages and may represent physiological changes induced in one or more of the three types present in great numbers in the earlier stages. One type might be characterized as a variant of *Bacillus subtilis*, the others might be considered variants of *Bacillus mesentericus* or *B. vulgatus*, possessing as they do some of the distinguishing characteristics of each of these species.

Members of the genus *Clostridium* are present on cured tobacco and may, under certain conditions, multiply rapidly, seriously affecting the quality of the finished product. Insufficient oxygen is responsible for the multiplication of these forms. The species of this genus found on fermenting tobacco include cellulose-decomposing types capable of reproduction at a temperature of 55° C. Rotting encountered during the fermentation may usually be attributed to members of this genus.

Gram-negative bacteria and non-spore forming rods have not been found reproducing in a satisfactory fermentation of cigar-leaf tobacco.

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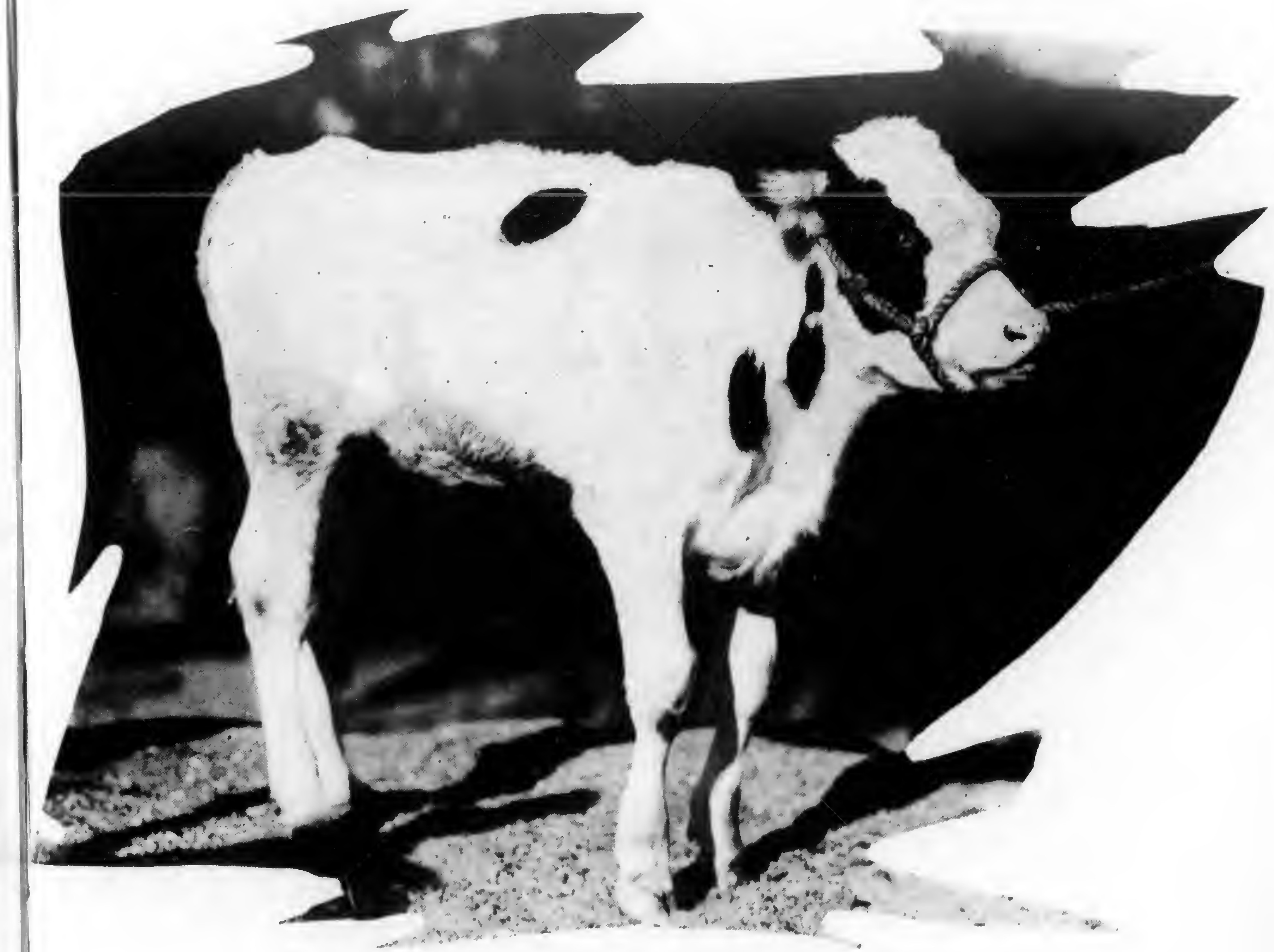
BULLETIN 364 JUNE 1938

The Vitamin D Requirement of Dairy Calves



Calf 415, control. This animal displays gross symptoms of rickets.

THE PENNSYLVANIA STATE COLLEGE
SCHOOL OF AGRICULTURE AND EXPERIMENT STATION
STATE COLLEGE, PENNSYLVANIA

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Standard Brands Incorporated, New York, N. Y., and National Oil Products Company, Harrison, N. J., made grants to the Pennsylvania Agricultural Experiment Station for research on calf rickets. These firms also supplied the yeast and cod-liver oil concentrate used in the experiment.

The carotene used was contributed by Purina Mills, St. Louis, Mo.

PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION

BULLETIN 364[*] STATE COLLEGE, PA. JUNE 1938

The Vitamin D Requirement of Dairy Calves[1]

S. I. BECHDEL, *Professor of Dairy Husbandry;* N. W. HILSTON, *Graduate Fellow in Dairy Husbandry;* N. B. GUERRANT, *Professor of Biological Chemistry; and* R. A. DUTCHER, *Professor of Agricultural and Biological Chemistry*

THERE is rather general agreement among dairy investigators and herdsmen that the average calf ration fed on dairy farms in the northern states should be fortified with vitamin D supplement. This is due chiefly to the fact that hays of poor quality are fed and weather conditions during the winter months are such that it is necessary to confine calves indoors almost continuously. Scientific literature gives little definite information on the number of units of vitamin D required per day for best growth and well being of dairy calves. Definite information on this phase of calf nutrition would aid materially in formulating calf starters and also in furnishing intelligent advice on the feeding of vitamin D supplements.

The investigation presented here was planned with three main objects in view: first, to determine the number of U.S.P. units of vitamin D required per day per 100 pounds live weight for growth and well being of calves from birth to 7 months of age; second, to compare the relative anti-rachitic effect of irradiated dry yeast and cod-liver oil concentrate on a U.S.P. unit basis with calves as laboratory animals; and third, to investigate the value of the x-ray in studying calcification of the bones of living calves.

REVIEW OF PREVIOUS RESEARCH

Between the years 1931 and 1933, the Pennsylvania Agricultural Experiment Station and other stations *(2, 12, 20)* published experimental data which proved that calves are susceptible to rickets, and that optimum skeletal growth and well being could not be expected with an inadequate supply of vitamin D in the diet.

Morrison and Rupel *(19)* reported that calves fed a legume hay of good quality received no benefit from exposure to sunlight. Bechtel et al. *(4)* have shown that corn cut for silage at the usual stage of maturity possesses definite anti-rachitic properties when fed to dairy calves. Duncan and Huffman *(6)* have presented experimental evidence that dairy calves have the ability to make use of solar ultraviolet radiation to increase the concentration of calcium and phosphorus of the blood serum.

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[*] Publication authorized April 1, 1938.

[1] The data presented in this bulletin are taken from a thesis submitted by N. W. Hilston to the Graduate School of The Pennsylvania Sate College in partial fulfillment of requirements for the degree of Doctor of Philosophy.

Hess et al. *(10)* reported that 60,000 rat units of vitamin D in the form of irradiated yeast imparted practically the same anti-rachitic value to milk as did 200,000 rat units in the form of viosterol when fed to dairy cows. By feeding high producing cows a normal ration plus 200 grams of irradiated yeast, Hart et al. *(7)* found that the calcium and phosphorus of the blood serum remained unchanged.

The work of Hart, Steenbock and Teut *(8)* indicates that the vitamin of cod-liver oil is poorly, if at all, absorbed from the intestinal tract of cows. Insko and Rupel *(15)* fed a ration of yellow corn, ground oats, bran, linseed-oil meal and skimmilk to dairy calves. Part of the calves also received cod-liver oil. These workers conclude that cod-liver oil has no value in a good standard ration for dairy calves. Hill *(11)* presented data showing that dairy cows are able to utilize the anti-rachitic factor in cod-liver oil. The work of Rupel, Bohstedt and Hart *(20)* also makes it evident that cod-liver oil and oxidized cod-liver oil are effective in preventing rickets in calves when adequate dosages are provided.

Wallis, Palmer and Gullickson *(21)* state that the average daily retention of calcium and phosphorus for normal calves is approximately 6.50 grams and 3.25 grams respectively. These workers report that calcium and phosphorus retention of vitamin D-deficient calves may be increased 14-fold in the case of calcium, and 11-fold in the case of phosphorus, by using vitamin D therapy. Increasing the mineral content of the ration fed to vitamin D-deficient calves has no favorable effect on the retention of minerals. According to Meigs et al. *(17)* the assimilation of calcium by dairy cows is likely to be greatly affected by any influence which causes them to go even slightly off feed.

Bechdel, Dutcher and Tucker *(1)* working with rachitic calves, found that treatment of the ration with ultraviolet light brought about an increase of 5 per cent in the deposition of ash in the ribs, femora and humeri. In 1930, Hill *(11)* reported that calves fed a rachitogenic ration and then exposed to ultraviolet rays showed improved calcification of the bones. Huffman, Duncan and Lightfoot *(14)* and Huffman and Duncan *(6, 13)* have shown that the eighth right rib of rachitic calves has a lower percentage of ash and of calcium and phosphorus than calves on a normal ration.

EXPERIMENTAL PROCEDURE IN 1935-36

Prophylactic technique was used for determining the vitamin D

requirement[2] (U.S.P. units[3]) for growth and well being of dairy calves from birth to 7 months of age. Irradiated yeast, oxidized cod-liver oil concentrate and unaltered cod-liver oil concentrate were used as vitamin D supplements.

Eighteen grade Holstein calves were used as experimental subjects. The calves were confined in a stable into which no direct sunlight was admitted. Each calf was stabled in a separate box stall 4 by 8 feet. Sawdust and wood shavings were used for bedding.

Whole milk was fed until the calves were 28 days of age when they were changed to reconstituted liquid skimmilk. A concentrate mixture was fed twice a day according to appetite. When the calves were able to eat about 3 pounds per day, reconstituted liquid skimmilk was discontinued and replaced with dry skimmilk powder.

Concentrate mixture.—The concentrate mixture which formed the basal diet was:

51.00 per cent yellow corn
20.00 per cent ground oats
20.00 per cent corn gluten meal
4.25 per cent linseed-oil meal
4.25 per cent soybean-oil meal
0.50 per cent sodium chloride

In addition to the skimmilk and concentrate mixture, night-harvested dehydrated alfalfa hay was fed with a maximum daily allowance of 1 pound per calf per day. Hay feeding was discontinued after 140 days to reduce the vitamin D potency of the basal ration which proved to be too high to permit the development of rickets.

All the animals were maintained on the basal diet for the first 40 days of the experiment. This dietary treatment was followed so that any possible vitamin D reserves of the calves would be reduced, as nearly as possible, to the same level. At the end of this period, the subjects were divided into comparable groups as to age, weight, sex and existing serum calcium and phosphorus, table 4.

The amount of supplement to be fed was calculated for a 28-day period on the basis of live weight plus half the gain during the preceding 28-day period. The required amounts of non-irradiated dry yeast, irradiated dry yeast, and cod-liver oil concentrate for each 28-day period was divided into approximately eight equal portions and these were put into one-half ounce gelatin capsules. This plan made it possible to feed the supplement twice a week. A "balling gun" was used to insert the capsule into the throat of the calf. This technique afforded complete assurance that no supplement would be lost in feeding. The loaded capsules were stored in individual containers and held at a temperature of -5° to -20° F until fed.

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[2] The method of determining the minimum amount of vitamin D to prevent the onset of rickets is designated as "prophylactic technique."

[3] The U.S.P. (United States Pharmacopoeia) unit is an arbitrary standard established by the Vitamin Standards Committee of the League of Nations Committee on Public Health.

Cod-liver oil concentrate in amounts necessary for two calves was oxidized for destruction of vitamin A by aeration with a suction pump for a period of 24 hours, at a temperature of 60° C. After a second period (48 hours) of aeration with the bottle of oil in a constant temperature oven held at 100° C, oxidation was considered complete. The oxidized oil was held in cold storage until fed.

Table 1.—Calcium and phosphorus content of basal diet, 1935-36.

	PER CENT ELEMENTAL CALCIUM	PER CENT ELEMENTAL PHOSPHORUS
Grain mixture	0.075	0.351
Skimmilk powder*	1.24	0.96
Whole milk*	0.12	0.09
Alfalfa hay	1.197	0.251

*From Morrison *(18)*.

Preparation of rachitogenic calf diet.—The concentrate mixture, skimmilk powder and hay were secured at one time. All feeds were thoroughly mixed to make sure of getting representative samples for bio-assays and chemical analyses.

The corn and oats were secured from the College farm and stored in dark bins. The alfalfa hay (first cutting) was cut after sundown and dehydrated in an artifical dryer to prevent exposure to sunlight after mowing. The dehydrated hay was stored in a covered loft.

Table 2.—Vitamin D content of basal diet and supplements fed, 1935-36.

Grain mixture	181	U.S.P. units per pound
Skimmilk powder	181	U.S.P. units per pound
Whole milk*	10	U.S.P. units per quart
Dehydrated alfalfa hay	150	U.S.P. units per pound
Non-irradiated dry yeast	0.8	U.S.P. units per gram
Irradiated dry yeast	2400	U.S.P. units per gram
Oxidized cod-liver oil concentrate	60	U.S.P. units per gram
Unaltered cod-liver oil concentrate	300	U.S.P. units per gram

*This figure was determined by averaging the vitamin D content of normal cows' milk during the winter months, from the work of Bechtel and Hoppert *(3)*.

All the skimmilk powder was from one source. It was processed from milk produced in January, 1936. Sufficient non-irradiated dry yeast and cod-liver oil concentrate for the experiment were secured from one source at the same time.

The concentrate mixture was mixed in lots of 1000 pounds on a clean floor to avoid contamination from the feed mixer. The basal diet and all supplements were analyzed and assayed, tables 1 and 2. The accepted standard (U.S.P.) technique was used in determining the vitamin D potency of the basal diet and the supplements.

Methods of sampling.—Daily feed consumption was carefully recorded. The calves were weighed at the same time each week. The height at withers, in centimeters, was taken every 28 days.

During the last two months of the 1935-36 experiment, periodic x-rays of the left tibia and radius were taken. The x-ray results of this year are not reported since the work was largely for the purpose of developing a satisfactory technique.

Blood samples were taken from the jugular vein of each calf, weekly, immediately before the morning feeding. These were used for inorganic calcium and phosphorus determinations on the serum. The samples were retained in a refrigerator until the serum calcium and inorganic phosphorus determinations were made the following morning.

The calcium content of the blood serum was determined by the Clark-Collip *(5)* modification of the Kramer-Tisdall *(16)* method. Inorganic phosphorus was determined by the method of Youngburg and Youngburg *(22)*.

Bone-ash determinations.—At the time of slaughter, the seventh, eighth, ninth and tenth left ribs were removed for bone-ash determination. The eighth and ninth ribs were placed in 95 per cent alcohol. The seventh and tenth were frozen and retained in storage in case repeated analyses should be desired. The eighth and ninth ribs were cleaned of all flesh after being in the alcohol 10 days.

The length of each rib from the proximal to the distal end over the greater curvature was measured with a flexible steel tape. The small cartilagenous cap on the distal end was included in this measurement. Ten per cent of the total length of each rib at the distal end was cut off with a hack saw. A second 10 per cent was then cut off as a separate sample. These two samples, the first and second 10 per cent of the total length, were designated as samples 1 and 2 respectively. The remaining 80 per cent of each rib (proximal end) was designated as sample 3.

The next step consisted in crushing each sample with pliers and grinding it in an iron mortar. Each ground sample was wrapped in two folds of No. 615 (25cm.) filter paper and tied securely with cord. The filter papers containing the samples were individually wrapped with cheesecloth and bound with copper wire. The bone samples so secured were placed in a large cheesecloth bag to facilitate handling them in a Lloyd Drug Extractor. All of the bone samples were extracted at the same time for a period of 8 hours with 95 per cent alcohol. After the alcoholic extraction the samples were allowed to dry over night at room temperature. The following day they were extracted for 8 hours with petroleum ether. Then the extraction was considered complete and the bones were dried over night in a constant temperature oven at 60° C.

The samples were placed into previously heated (530° C) and weighed crucibles. These were put in a constant temperature oven and held for 12 hours at 100° C. The samples were then cooled, weighed and placed in an electric muffle furnace. The heat was turned on gradually so that about 2 hours were required to attain a temperature of 530° C. The samples were held at this temperature for 12 hours to insure complete ashing. They were cooled in a dessicator and final weighings made, table 6.

Table 3.—Calcium intake by periods for calf 368.

PERIOD	DURATION OF PERIOD Days	DAILY AVERAGE ELEMENTAL CALCIUM INTAKE PER 100 POUNDS LIVE WEIGHT Grams
1	36	7.5
2	28	7.5
3	28	6.5
4	28	5.1
5	28	3.0
6	41	0.7
7	28	0.6
8	38	0.9
9	41*	0.9
10	40†	8.5
11	16	9.2
Total	352	Average 3.8

*Drenched with 10 gm. of precipitated chalk; 80 cc. of 23 per cent calcium gluconate injected subcutaneously. The calcium of the calcium gluconate is not included in the above figures. The product was obtained from Jen-Sal Laboratories, Kansas City, Mo.

†Changed to new basal diet which contained 2 per cent calcium carbonate.

DISCUSSION OF EXPERIMENTAL RESULTS, 1935-36

A summary of the data obtained in the experiments conducted in 1935-36 appears in tables 4, 5 and 6. Extensive data on inorganic calcium and phosphorus of the blood are omitted for the sake of brevity. These tables reveal a rather inconsistent picture. A detailed report on one subject, calf 368, best explains the disappointing results of the first year of the study. This animal received 300 U. S. P. units of vitamin D from oxidized cod-liver oil concentrate. He was kept on experiment until 357 days old. When he was 190 days of age the oxidized cod-liver oil concentrate was replaced with untreated cod-liver oil concentrate in amounts to furnish 1500 U.S.P. units of vitamin D per day. This higher level of supplement feeding was continued to the end of the experiment. When the calf was 113 to 190 days of age the inorganic blood calcium was below the normal level. The inorganic phosphorus was below normal almost continuously throughout the experiment.

The alfalfa hay and skimmilk powder were left out of the basal diet after about 140 days of the experiment. This was done to reduce the vitamin D potency of the total ration. Unfortunately, the removal of these constituents reduced the elemental calcium intake so low that this individual and others on the experiment were not receiving the proper amount of this element necessary for growth and well being. During the first 148 days of the experiment the calcium intake for this calf per 100 pounds live weight varied from 3.0 to 7.5 grams per day. This amount was barely sufficient to take care of nutritional requirements. When the hay and skimmilk powder were left out of the diet, the daily calcium intake dropped to an average of 0.6 grams per day per 100 pounds live weight, table 3. Obviously, this amount was entirely too low. This is typical of what took place with all the calves on the experiment.

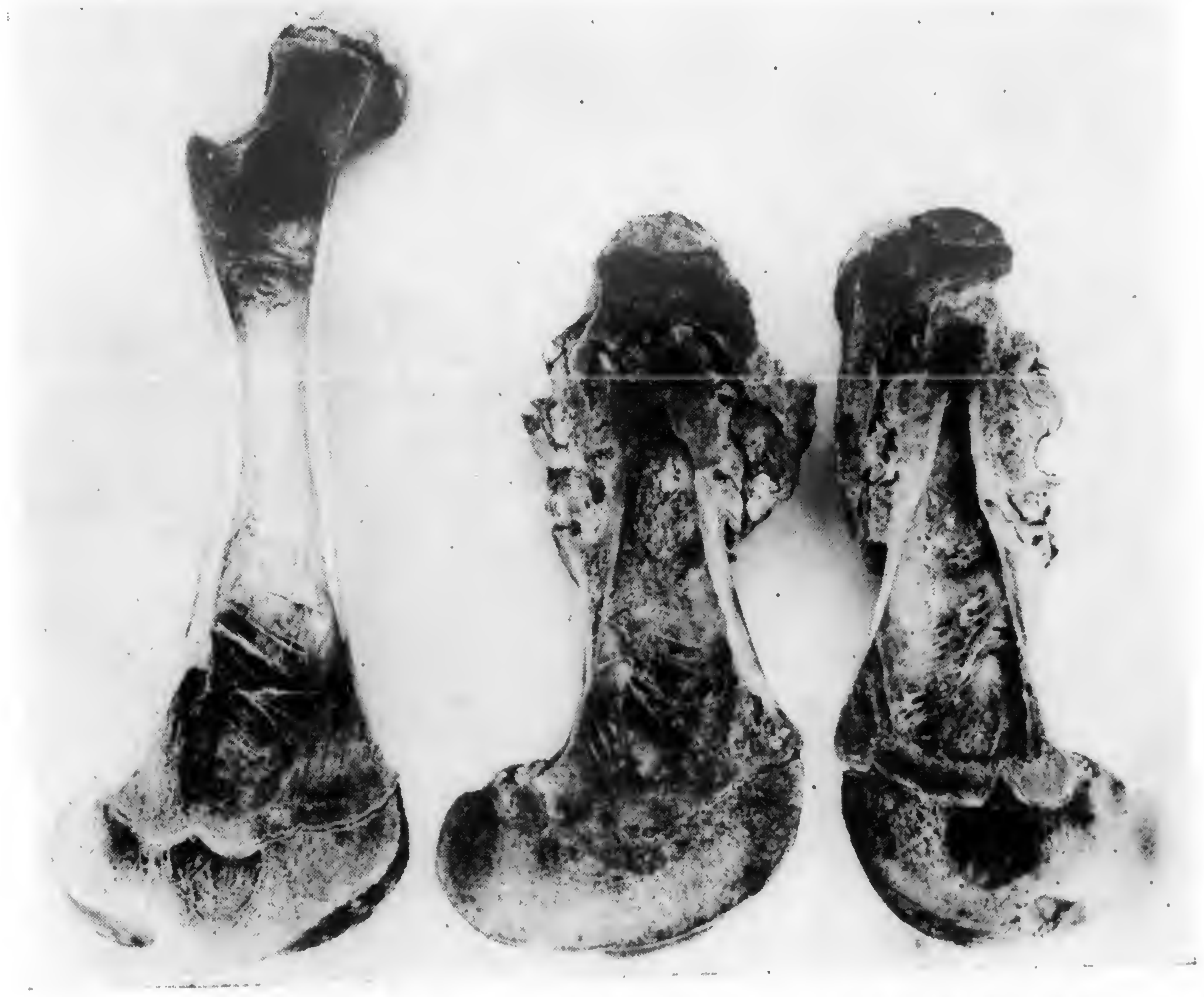

Fig. 1.—Comparison of a normal femur from calf 378 and the broken femora from calf 368. The two on the right had broken, telescoped and healed. These bones were taken from animals of the same age.

About 35 days after hay and skimmilk powder were removed from the basal diet, calf 368 became so lame that he was unable to get on his feet. In a few days his condition became so severe that he could not rise even when assisted. However, his appetite remained normal. At this time he was receiving 300 U.S.P. units of vitamin D from oxidized cod-liver oil concentrate. Ten days before this difficulty occurred he was put on curative treatment, the oxidized cod-liver oil being replaced with 1500 U.S.P. units in untreated cod-liver oil concentrate. It was evident that there was no shortage of vitamin D. None of the calves developed gross symptoms of rickets.

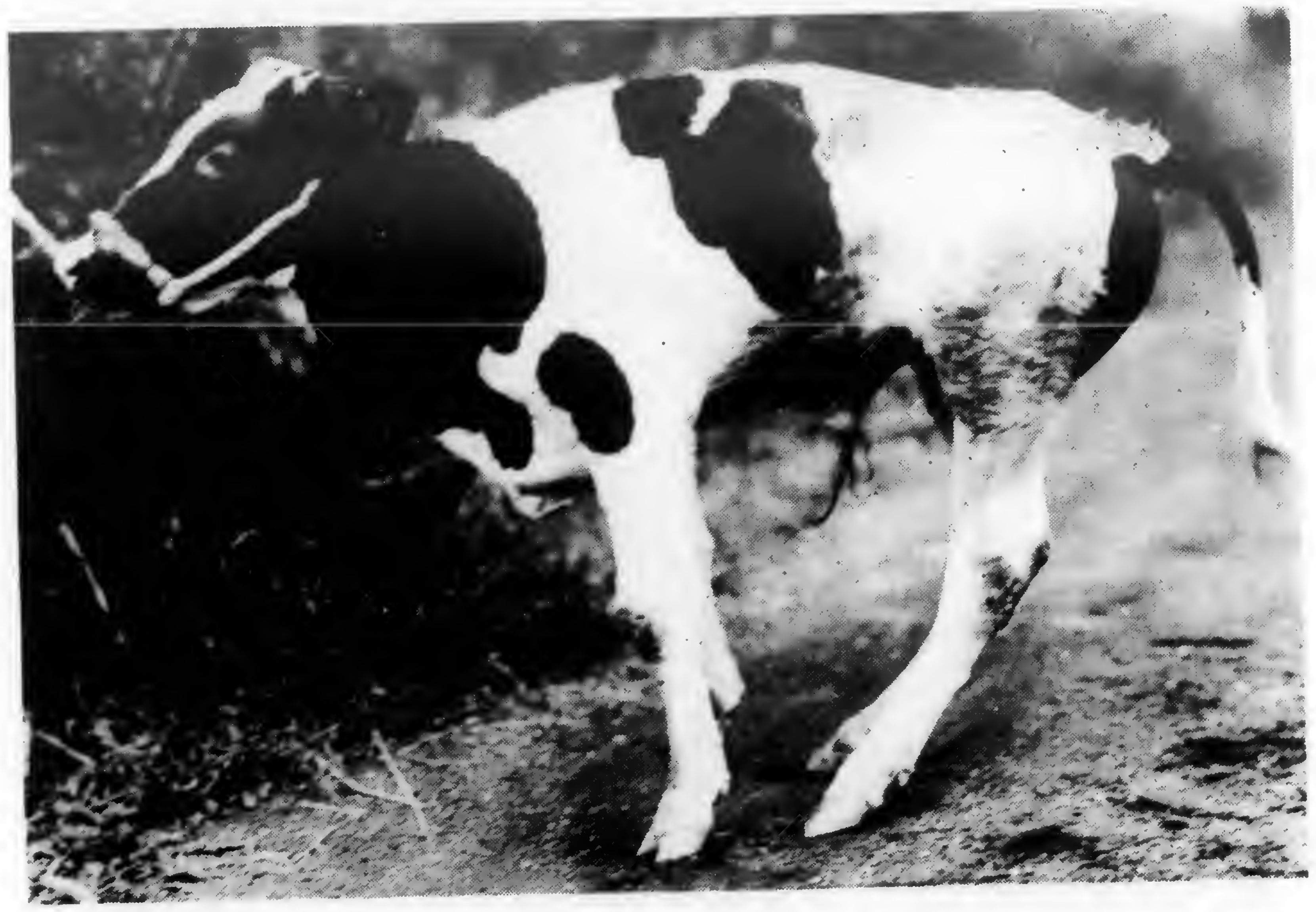

Fig. 2.—Calf 368, showing the characteristic standing position after regaining the use of its legs. Photograph taken at 387 days of age.

Although it was not known at the time, the inability of this calf to stand up was caused by a fracture of the femur bones at the narrowest point in the shaft near the proximal end. The shaft actually telescoped into the proximal end, fig. 1. Possibly the very high level of vitamin D (1500 U.S.P. units from cod-liver oil concentrate) may have supplied a sparing action which promoted healing despite the low calcium intake.

This calf finally improved to the point where it was able to get on its feet, after being down for 57 days. The photograph taken at this time, fig. 2, indicates that the femora had shortened and pulled the rump into a characteristic position. The calf probably stood in this position also as a means of protection, or perhaps the position afforded less pain or discomfort.

Little or no improvement followed, so about 42 days after he was first able to get on his feet the calcium in the diet was raised from .9 gram per 100 pounds per day to 8.5 grams, table 3. Improvement then was very rapid. In a few days he was able to get up and down with very little difficulty, and he soon became active in every way. The rump began to straighten; the photograph, fig. 3, which was taken after 56 days on the high calcium level, indicates a normal top line. The animal appeared normal in every respect at this time.

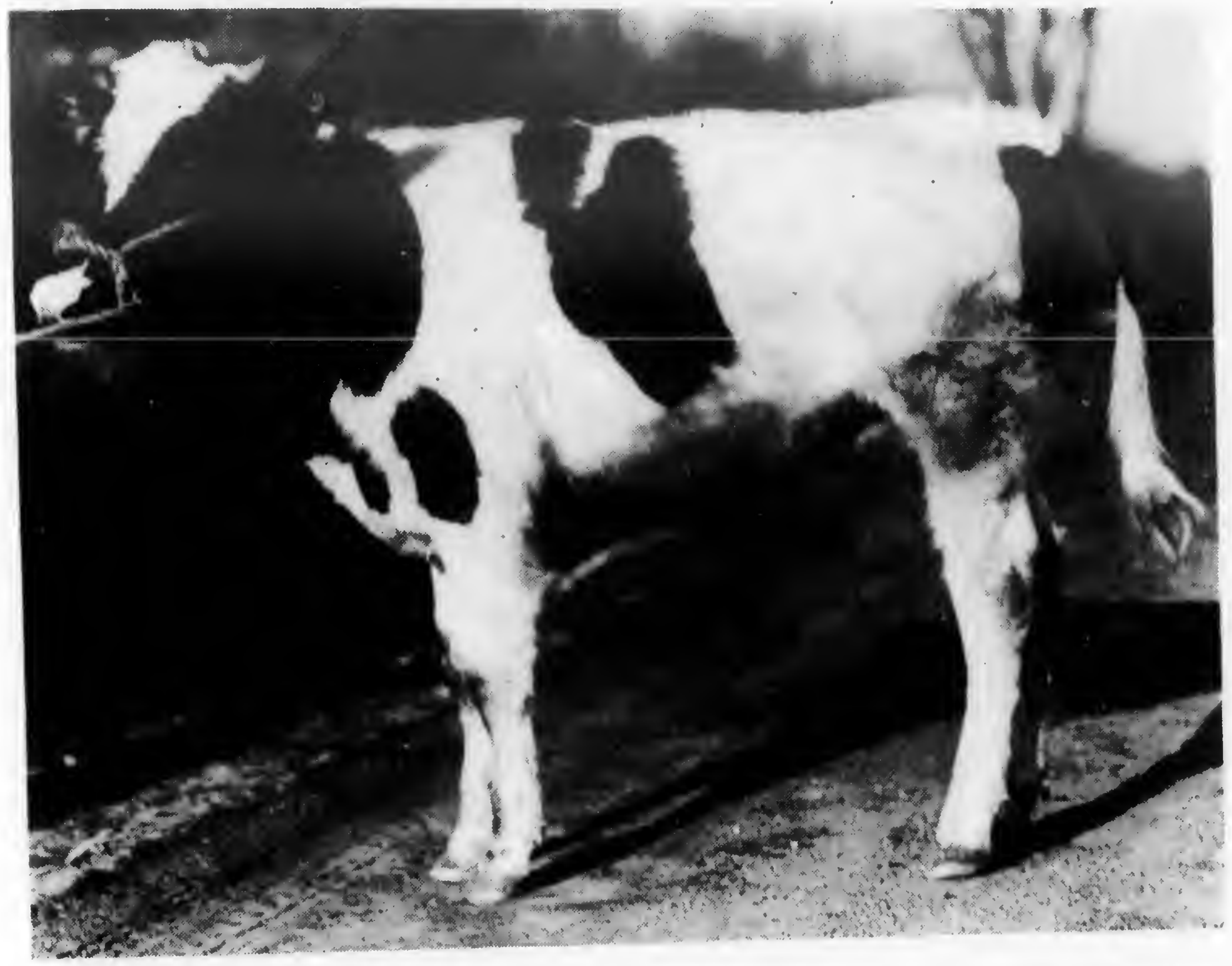

Fig. 3.—Calf 368. This animal was able to stand in a fairly normal position at 357 days of age, after the femora had broken and healed. Compare figure 2.

When this calf was slaughtered both femora were found to have been broken and to have healed in practically the same manner. The longitudinal sections, fig. 1, indicated heavy deposition of bone tissues around the fracture. No doubt the greater part of this healing took place during the last 56 days on the high calcium diet.

The bone-ash data gave a very inconsistent picture, table 6. There was no evidence that one vitamin level was appreciably more effective than any other in promoting bone-ash deposition. The 12 calves on which bone-ash data were secured were slaughtered between the ages of 183 and 204 days. If these animals had been continued on experiment several months longer, as in the case of calf 368, probably other cases of bone fractures would have occurred. Calf 370, which was virtually a control animal, also developed a

Table 4.—Summary of data, 1935-36.

Calf No.	Sex	Level of Supplement Fed per Day per 100 Pounds Live Weight U.S.P. units	Duration of Experimental Period days	Age at End of Experimental Period days	Initial Weight lbs.	Final Weight lbs.
371	Male	Control	157	170	73	223
381	Male	Control	180	190	117	372
370	Female	Non-irradiated years in amounts equivalent to amount of irradiated yeast furnishing 2500 units..................	162	175	66.5	244
384	Male	Controls	180	188	123	271
378	Female	125 irradiated yeast	180	181	96	321
386	Male	125 irradiated yeast	180	194	111.5	342
382	Male	125 cod-liver oil	180	193	119	331
385	Male	250 irradiated yeast	180	187	107	346
387	Male	250 cod-liver oil	180	182	113	351
376	Female	500 irradiated yeast	180	196	160	321
375	Female	500 cod-liver oil	180	196	101.5	320
377	Female	1500 irradiated yeast	180	188	110	324
380	Female	1500 irradiated yeast	180	183	94	316
379	Female	1500 cod-liver oil	180	184	100	360
383	Male	2500 irradiated yeast	180	185	90	344
367	Male	2500 cod-liver oil	180	181	91	345
369	Male	50 oxidized cod-liver oil	164	177	90	265
368	Male	300 oxidized cod-liver oil	176	176	80	281

broken femur at 283 days of age and died 5 days later. She had been changed to curative treatment (2500 units level of irradiated yeast) at 189 days of age. The blood calcium had been consistently below normal after she was 67 days of age, but returned to normal after 23 days of the yeast feeding. It is evident that the low calcium in the diet was responsible for the fractured femur. This case parallels that of calf 368.

Table 5.—Summary of data, 1935-36.

Calf No.	Initial Height at Withers Cms.	Final Height at Withers Cms.	Approximate Number of Units Fed per Day per 100 Pounds Live Weight (Includes Diet and Supplement) U.S.P. Units	Average Daily Elemental Calcium Intake per 100 Pounds Live Weight Cms.	Average Daily Elemental Phosphorus Intake per 100 Pounds Live Weight Cms.
371	70	91.5	435	4.1	4.6
381	81.5	102	295	3.0	3.1
370	70	92	425	3.9	4.5
384	83	98	335	3.7	3.7
378	76	95	390	3.2	3.4
386	81.5	103	410	3.2	3.4
382	81.5	101.5	420	3.2	3.4
385	81	101	490	3.2	3.4
387	80	99	495	3.1	3.4
376	77	97	705	3.4	3.6
375	76	98.5	695	3.5	3.6
377	81	102	1365	3.3	3.5
380	79	96	1380	3.4	3.6
379	78.5	98.5	1325	3.2	3.3
383	78.5	100	2060	3.3	3.5
367	78	101.5	2060	3.4	3.4
369	77.5		380	3.5	3.7
368	73.5	97.5	595	3.8	4.2

All things considered, the bone-ash results are about what might be expected. There was virtually no shortage of vitamin D in the control diet. The different levels of vitamin D supplied in irradiated dry yeast and cod-liver oil concentrate represented different levels of excess vitamin D. It would not be reasonable to expect normal deposition in any of the groups when the amount of calcium was so far below what is generally considered to be the optimum for growth and well being.

Table 6.—Bone-ash data, 1935-36.

	Ash Content of Eighth Left Rib, Per Cent				Ash Content of Ninth Left Rib, Per Cent			
Calf No.	First 10% of Distal End	Second 10% of Distal End	Remaining 80%	Total	First 10% of Distal End	Second 10% of Distal End	Remaining 80%	Total
371	24.75	42.19	50.64	45.30	28.12	47.68	51.70	47.76
381	31.80	43.96	49.39	45.93	34.29	42.08	51.62	48.10
Controls								
370*								
384	22.36	36.95	48.16	42.43	33.29	39.58	50.40	46.90
Controls								
378†								
386	27.07	47.94	52.26	48.83	32.84	51.08	52.94	50.02
382	32.10	51.86	52.35	49.34	33.33	46.77	52.73	49.51
125 unit level								
385	31.95	43.00	48.17	45.61	28.64	44.31	51.03	46.57
387	31.24	46.08	48.84	46.34	37.50	44.12	49.25	47.53
250 unit level								
376	28.44	47.17	53.30	49.45	36.44	52.18	52.69	50.88
375‡								
500 unit level								
377‡								
380	27.25	40.98	51.09	46.96	34.49	48.26	51.12	49.14
379‡								
1500 unit level								
383	29.87	41.84	49.14	45.59	31.25	45.31	50.26	47.20
367	28.60	46.86	52.19	48.63	33.96	49.53	51.48	48.99
2500 unit level								
369	32.62	44.84	47.84	44.83	35.67	43.46	49.74	47.00
368*								
Oxidized cod-liver oil								

*Put on curative treatment, not slaughtered.
†Changed to control, not slaughtered.
‡Sold, not slaughtered.

The usual gross symptoms, as reported previously *(2)* did not appear in the controls or in any of the individuals on the different levels of vitamin D. The bones of the control animals were appreciably softer than the bones of those on the different levels of vitamin D supplement. However, none of the calves, even those on the highest levels, showed a hardness of bone equal to that of a normal calf.

The blood calcium values were commonly low throughout the experiment, especially in the control calves and those on the lower levels of vitamin D. Considerable fluctuation from below to above normal value in calcium and, to some extent, phosphorus was common throughout the experiment in all of the groups. In general, the blood value did not present a consistent picture.

It is evident that authentic data on the vitamin D requirement for growth and well being could not be expected under the conditions that existed in the experimental set-up for 1935-36. An error was made in planning the diet; the calcium content was too low. This was further complicated by the fact that the feeds selected for the basal diet proved to be higher in vitamin D than was anticipated.

The data secured in the first year of this study are, therefore, of little value in solving the problem of vitamin D unit requirements. This work had real value, however, in perfecting technique and in paving the way for the more completely controlled experiment of 1936-37. Furthermore, the data, although inconsistent in many respects, did indicate quite clearly that the unit requirement was considerably less than had been anticipated, and that it would not be necessary to include levels higher than 500 units per 100 pounds of live weight per day in planning the succeeding experiment.

In the late months of the experiment, x-ray technique in the study of rickets over a prolonged period was studied. A satisfactory technique was worked out and was adopted in the second year of the study.

EXPERIMENTAL PROCEDURE IN 1936-37

The procedure in the second year was much the same as that of the first year. Certain changes were made in the light of results already obtained. Prophylactic technique was used to determine the U.S.P. unit requirement of vitamin D for growth and well being of dairy calves from birth to 7 months of age. Irradiated dry yeast and cod-liver oil concentrate were used again as the sources of vitamin D supplement.

Fifteen grade Holstein calves were used as experimental subjects. All of them were less than 2 weeks of age when placed on experiment. They were confined in the stalls that were used during the previous year. The stable was made completely dark by covering the windows with plyboard. Electric light was used for short periods whenever it was necessary to feed or work with the calves in any way. The average length of time the lights were burned was about 1 hour per day. Other stable improvements included a forced-air ventilation system, a hot-water heating plant and a concrete floor. A sewer drain was laid in each stall to provide clean, dry beds for the calves at all times. These improvements made it possible to keep the temperature of the stable at 50° F and at the same time have plenty of fresh air.

The method of feeding was almost exactly the same as that employed during the previous year except that practically no alfalfa hay was fed. The hay was replaced with domestic dried sugar beet pulp which was fed with a maximum daily allowance of 2 pounds per calf.

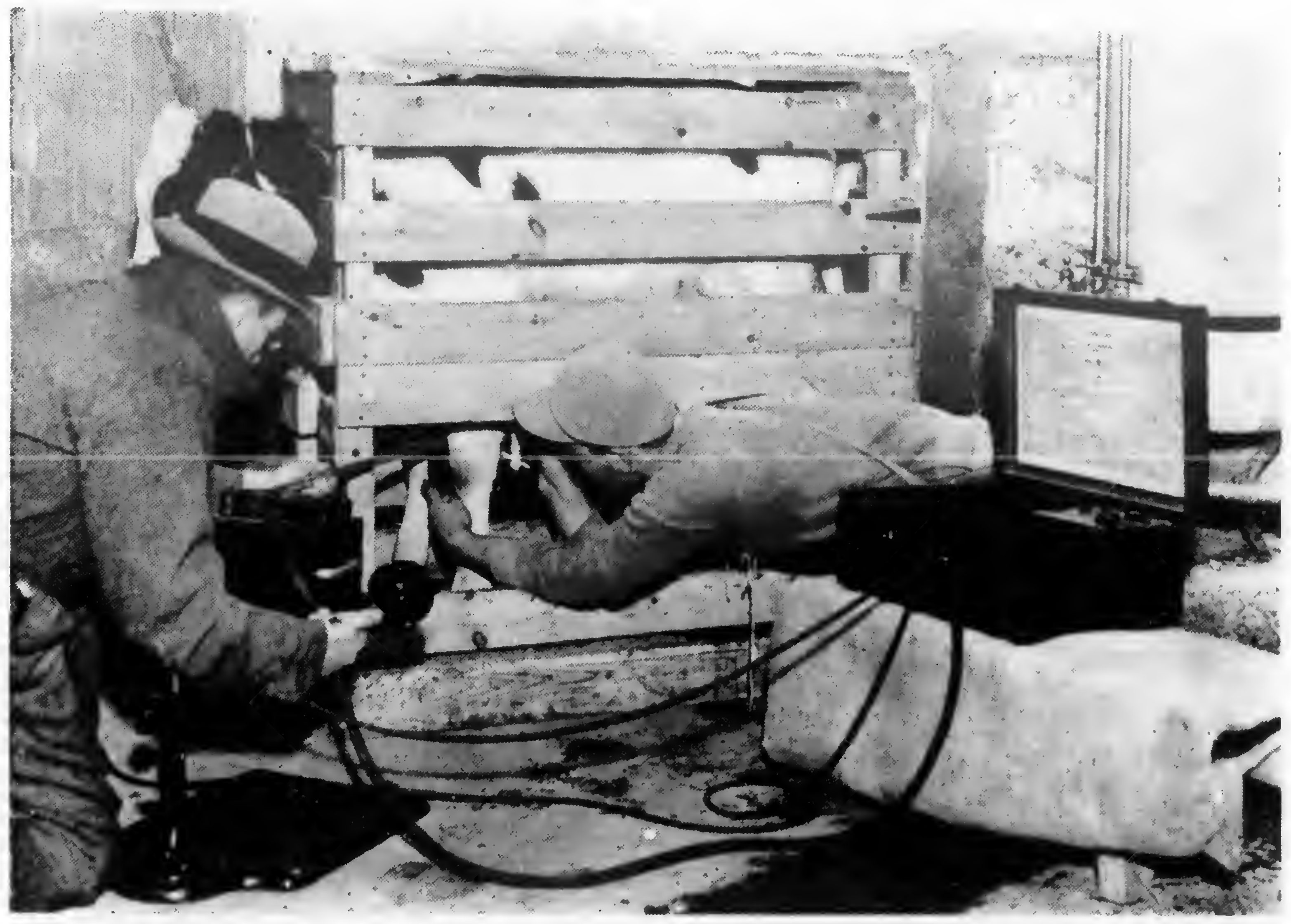

Fig. 4.—Technique employed in securing periodic x-rays of distal end of left ulna. The calf is held securely in a crate to avoid motion. All x-rays were taken at a uniform distance of 30 inches between the tube and cassette.

Basal ration.—The concentrate mixture was:

50.0 per cent yellow corn
20.0 per cent ground oats
20.0 per cent corn gluten meal
7.5 per cent soybean oil meal
2.0 per cent calcium carbonate
0.5 per cent sodium chloride

This basal ration mixture differed from that used the previous year mainly in its calcium content. All the calves were maintained on the basal diet for the first 42 days. This was followed in order that all of the animals might be depleted, as nearly as possible, to the same plane of nutrition when vitamin D supplements were added to the basal diet at various levels. At the end of this period the subjects were divided into comparable groups as to age, weight, sex, inorganic calcium and phosphorus in the blood and x-ray data, table 9.

The supplements were calculated, weighed, stored and fed in the same manner as in 1935-36. The concentrate mixture, skimmilk powder and beet pulp were all secured at one time. All feeds were thoroughly mixed in order to be assured of representative samples for bio-assays and analyses. The corn was purchased in September 1936, but was from the crop of the previous year. Old corn has a lower vitamin D content than new crop corn. It was assayed for vitamin D potency to make certain that the concentrate mixture was sufficiently low to permit gross development of rickets in the control animals.

Table 7.—Elemental calcium, magnesium, and phosphorus content of basal diet, 1936-37.

	PER CENT CALCIUM	PER CENT MAGNESIUM	PER CENT PHOSPHORUS
Grain mixture	0.871	0.171	0.29
Skimmilk powder	1.265	0.189	0.931
Whole milk*	0.12		0.09
Alfalfa hay	1.184	0.373	0.267
Beet pulp	0.68	0.444	0.07

*From Morrison *(18)*.

The plan called for the use of night-harvested dehydrated alfalfa hay as a source of vitamin A. Unfortunately it could not be fed in appreciable amounts in the basal diet since its vitamin D potency was much higher than anticipated, table 8. The maximum amount fed to a single calf did not exceed 1.6 pounds over the entire experimental period. Domestic dried sugar beet pulp was used instead of the alfalfa hay as the sole source of roughage in the later months of experiment. The bio-assay, table 8, reveals that the vitamin D potency of the beet pulp was almost negligible. A commercial carotene preparation was fed to all calves to make certain of no deficiency of vitamin A. The carotene was measured into half-ounce capsules and fed weekly with the aid of a balling gun. The amount of vitamin A fed was approximately 7500 U.S.P. units per calf per day.

Table 8.—Vitamin D content of basal diet and supplements fed, 1936-37.

Grain mixture	60 U.S.P. units per pound
Skimmilk powder	200 U.S.P. units per pound
Dehydrated alfalfa hay (harvested at night)	300 U.S.P. units per pound
Beet pulp	40 U.S.P. units per pound
Whole milk*	10 U.S.P. units per quart
Irradiated dry yeast	2,400 U.S.P. units per gram
Cod-liver oil concentrate	300 U.S.P. units per gram

*This figure was determined by averaging the vitamin D content of normal cows' milk during the winter months, from the work of Bechtel and Hoppert *(3)*.

All the skimmilk powder was secured from one source. It was made from milk produced in a drought area of New York during August, 1936. The irradiated dry yeast and cod-liver oil concentrate were from the same lots as used in the previous experiment. Representative samples of these supplements were again subjected to bio-assay, table 8.

The concentrate mixture was prepared in lots of 1000 pounds. It was mixed on a clean floor, in order to avoid contamination that might occur in the use of a feed mixer. The basal diet and all supplements were analyzed and were bio-assayed for vitamin D content, as in the previous year, tables 7 and 8.

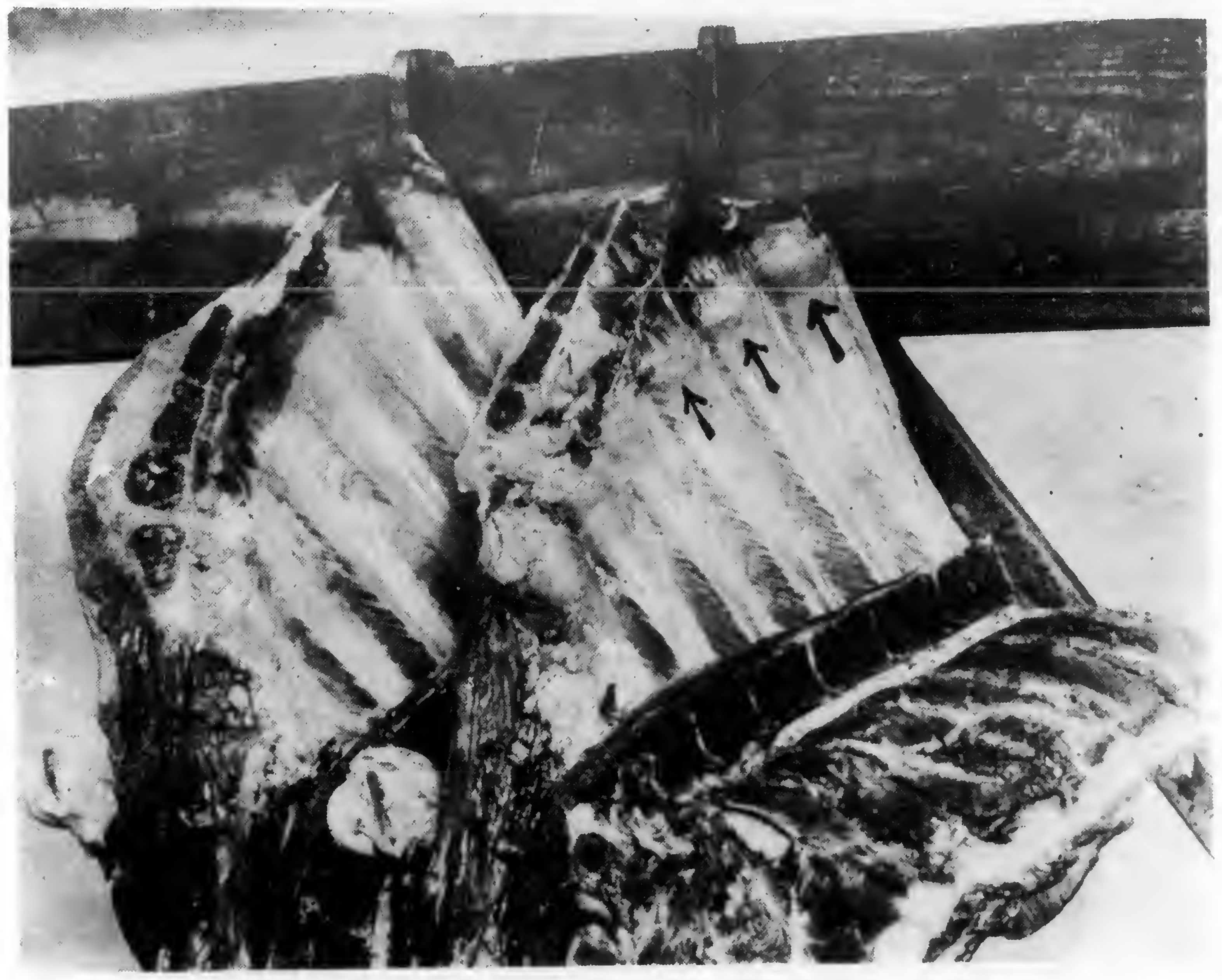

Fig. 5.—First to sixth ribs from twin calves slaughtered at 191 days of age. Calf 408 (left) received 500 U.S.P. units of vitamin D from irradiated yeast. Calf 409 (right) was a control animal. Note beading, distal end of ribs, on calf at right, as indicated by arrows. This was typical of the control group and to some extent of calves on the 100 unit level.

The feed consumption, serum calcium and phosphorus, weights and heights at withers were obtained and recorded after the procedure used in the previous year.

X-ray technique.—X-ray photographs of the distal end of the left ulna were taken at intervals of 2 weeks. The apparatus used was a Model F General Electric portable set. The 5 by 7-inch film was convenient and satisfactory.

In the preliminary work done late in the previous year, it was found that the width of the epiphyseal cartilage of the ulna was correlated with the results of inorganic calcium and phosphorus in the development of rickets, fig. 6. This led to the decision to take x-ray pictures of the distal end of the ulna every 2 weeks.

The calves were confined and securely fastened in a close-fitting crate to prevent movement at the time the picture was taken. It was necessary to expose the film in such a manner that the radius would not overshadow the ulna. By placing the x-ray tube at about a 75 degree angle to the anterior view of the leg, it was possible to photograph the ulna with very little interference from the radius, fig. 4. In the preliminary work it was found that a distance of 30 inches between the tube and film and an exposure of one-half to three-quarters of a second gave the best results. These specifications were followed throughout the experimental period.

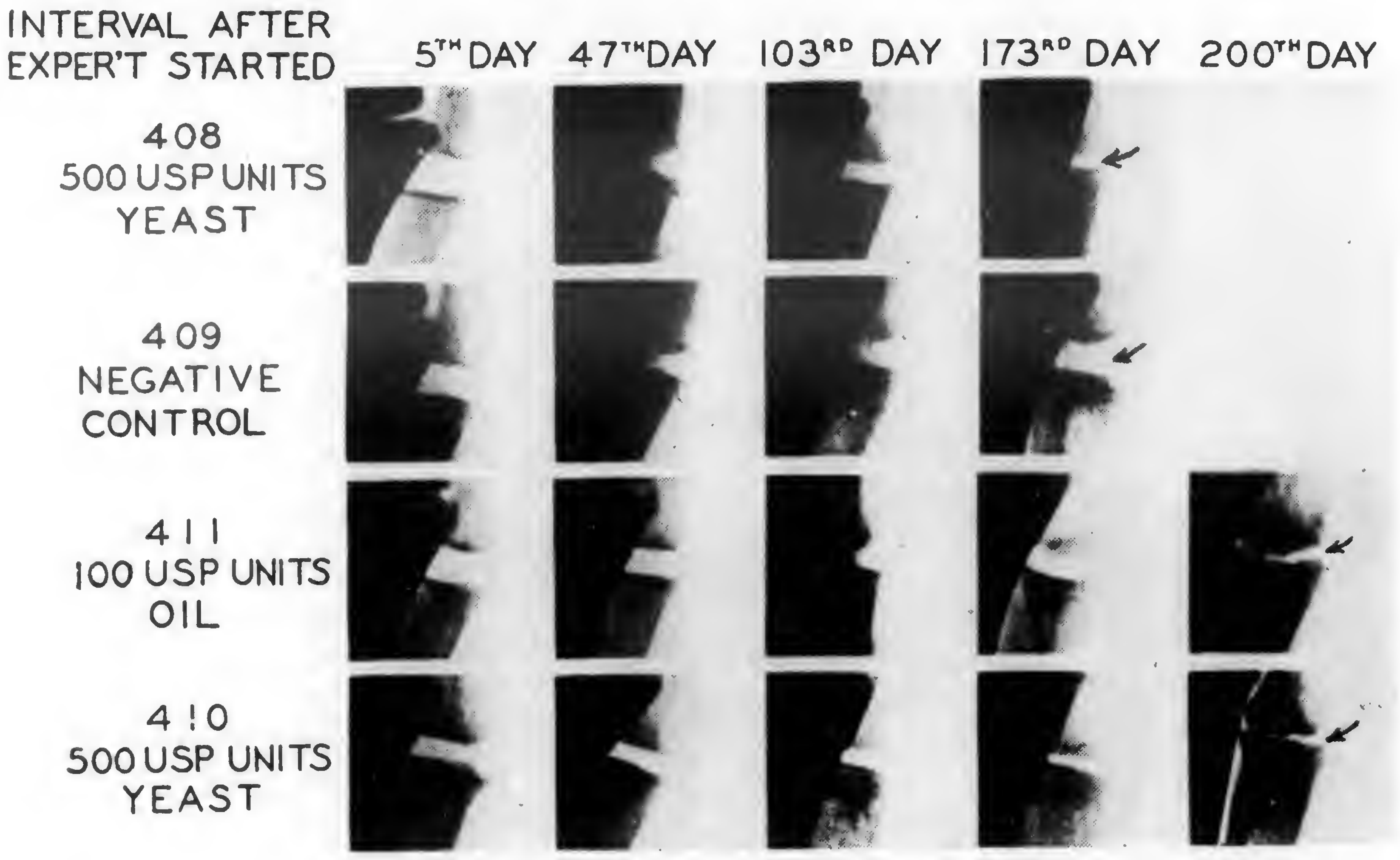

Fig. 6.—Showing the progressive narrowing of the epiphyseal cartilage in calves 408, 410 and 411, as compared with the negative control 409. Compare figure 7.

Bone-ash determination.—The procedure for the determination of bone ash was practically the same as that employed during the preceding year, with a few modifications. The eighth left rib only was used for this determination. The seventh left rib was used for making line tests. The ninth and tenth left ribs were frozen and placed in storage in case it would be necessary to repeat either of the above tests. Instead of placing the seventh and eighth ribs into 95 per cent alcohol, a 50 per cent alcohol solution was used after the standard technique for ash determinations on chicken bones.

The cartilagenous cap on the distal end of the rib was removed. This was done because the "cap" on different calves varied greatly in size and also seemed to contain no oestoid tissue. After the cartilagenous cap was removed, the ribs were measured, sectioned, ground and prepared for extraction as in the previous year.

The alcoholic and ether extractions were performed the same as in the previous year, except that 12 hours of extraction with each fat solvent were used instead of 8. The period was lengthened to be sure that all of the fatty material would be removed before ashing. It was thought that this technique would afford a more clear-cut distinction between various levels of vitamin D feeding than otherwise would be possible.

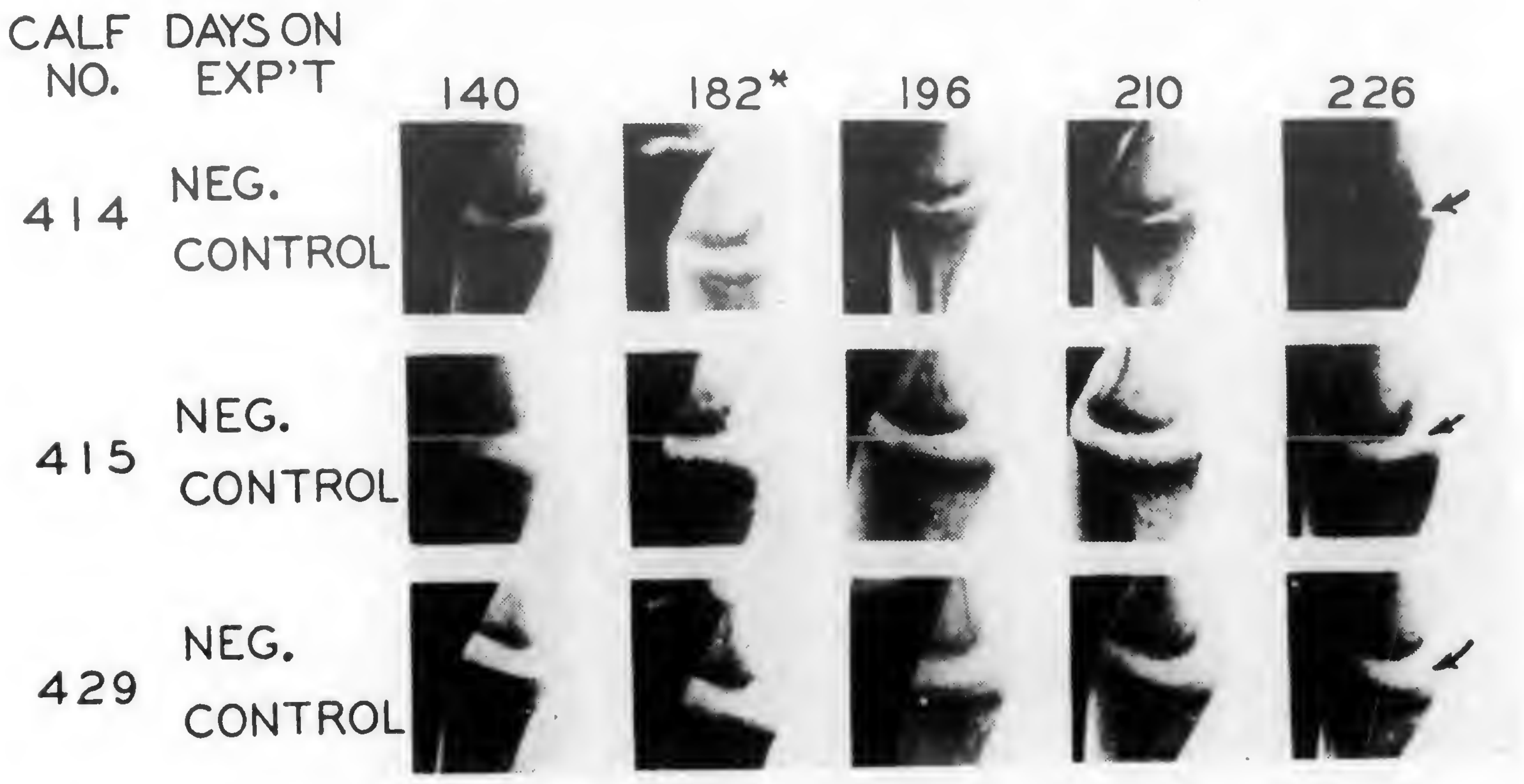

Fig. 7.—Showing progressive narrowing of epiphyseal cartilage of calf 414 after 182nd day, as compared with the two other controls. Calf 414 received 500 U.S.P. units of vitamin D per day in irradiated yeast per 100 pounds body weight, beginning on the 182nd day.

The method of drying, weighing and ashing were the same as in the previous year.

Line test.—Immediately after slaughter the seventh left rib was cleaned of all flesh, but the cartilagenous cap was not removed. The ribs were then placed in a 50 per cent solution of alcohol. After being in the alcohol for about 2 weeks, the ribs were removed and a 2-inch longitudinal section of the distal end was made with a hack saw. The cut sections were washed with distilled water. A 1 per cent solution of silver nitrate was used for the line test. After the bones had been immersed in this solution and the degree of calcification had developed, the ribs were photographed. The line test of the seventh rib was very satisfactory, but faulty photographic technique made it necessary to repeat the work with the ninth left rib.

Table 9.—Summary of data, 1936-37.

CALF No.	SEX	U.S.P. UNITS FED PER DAY PER 100 POUNDS LIVE WEIGHT	DURATION OF EXPERIMENTAL PERIOD Days	AGE AT END OF EXPERIMENTAL PERIOD Days	INITIAL WEIGHT Lbs.	FINAL WEIGHT Lbs.
409*	Female	Control	182	190	70.5	176
414	Female	Control	216	226	111.5	226
415	Female	Control	216	226	101.5	282.5
424	Female	Control	205	209	98	278
429	Female	Control	188	191	101	229
413	Female	100 irradiated yeast	216	223	95	366
419	Female	100 irradiated yeast	216	228	85	251
411	Female	100 cod-liver oil	216	220	85.5	299
417	Female	100 cod-liver oil	216	226	101.5	300
416	Female	300 irradiated yeast	216	225	101.5	301
423	Female	300 irradiated yeast	210	213	78	286
412	Female	300 cod-liver oil	216	224	111.5	287.5
421	Female	300 cod-liver oil	224	224	77.5	265
408*	Male	500 irradiated yeast	182	190	88	268
410	Female	500 irradiated yeast	216	225	128	381.5

*Calves 408 and 409 were twins.

The ninth ribs were taken from cold storage, thawed and placed in 95 per cent alcohol. After being in alcohol for 24 hours, the bones were next placed in petroleum ether for 24 hours. When the ether treatment was completed, another treatment of 24 hours with 95 per cent alcohol was used. The alcohol and ether treatments were used to remove as much fat from the bones as possible. The ribs were next sectioned, treated with silver nitrate and photographed, fig. 8.

Table 10.—Summary of data, 1936-37.

CALF No.	INITIAL HEIGHT AT WITHERS Cms.	FINAL HEIGHT AT WITHERS Cms.	APPROXIMATE NUMBER OF U.S.P. UNITS FED PER DAY PER 100 POUNDS LIVE WEIGHT (FROM DIET AND SUPPLEMENT)	AVERAGE DAILY ELEMENTAL CALCIUM INTAKE PER 100 POUNDS LIVE WEIGHT Grams	AVERAGE DAILY ELEMENTAL PHOSPHOROUS INTAKE PER 100 POUNDS LIVE WEIGHT Grams
409	69.5	83.3	180	10.2	3.7
414	77.8	92	205*	7.4	2.7
415	79.3	96.7	135	8.0	2.8
424	77.5	99	125	7.4	2.6
429	75.3	89.5	135	8.5	2.9
413	77	101.8	190	7.0	2.3
419	77.5	93.8	205	7.9	2.8
411	77.5	97.3	170	7.8	2.7
417	79	101.8	210	7.7	2.6
416	75.8	99.5	355	7.3	2.5
423	71	95	370	7.6	2.6
412	77	96.4	345	6.6	2.4
421	71.8	91.5	350	7.3	2.7
408	71.5	92.5	515	7.5	2.8
410	81.5	103.7	450	6.1	2.1

*Irradiated yeast (500 U.S.P. units daily) added to diet in last 28 days of feeding.

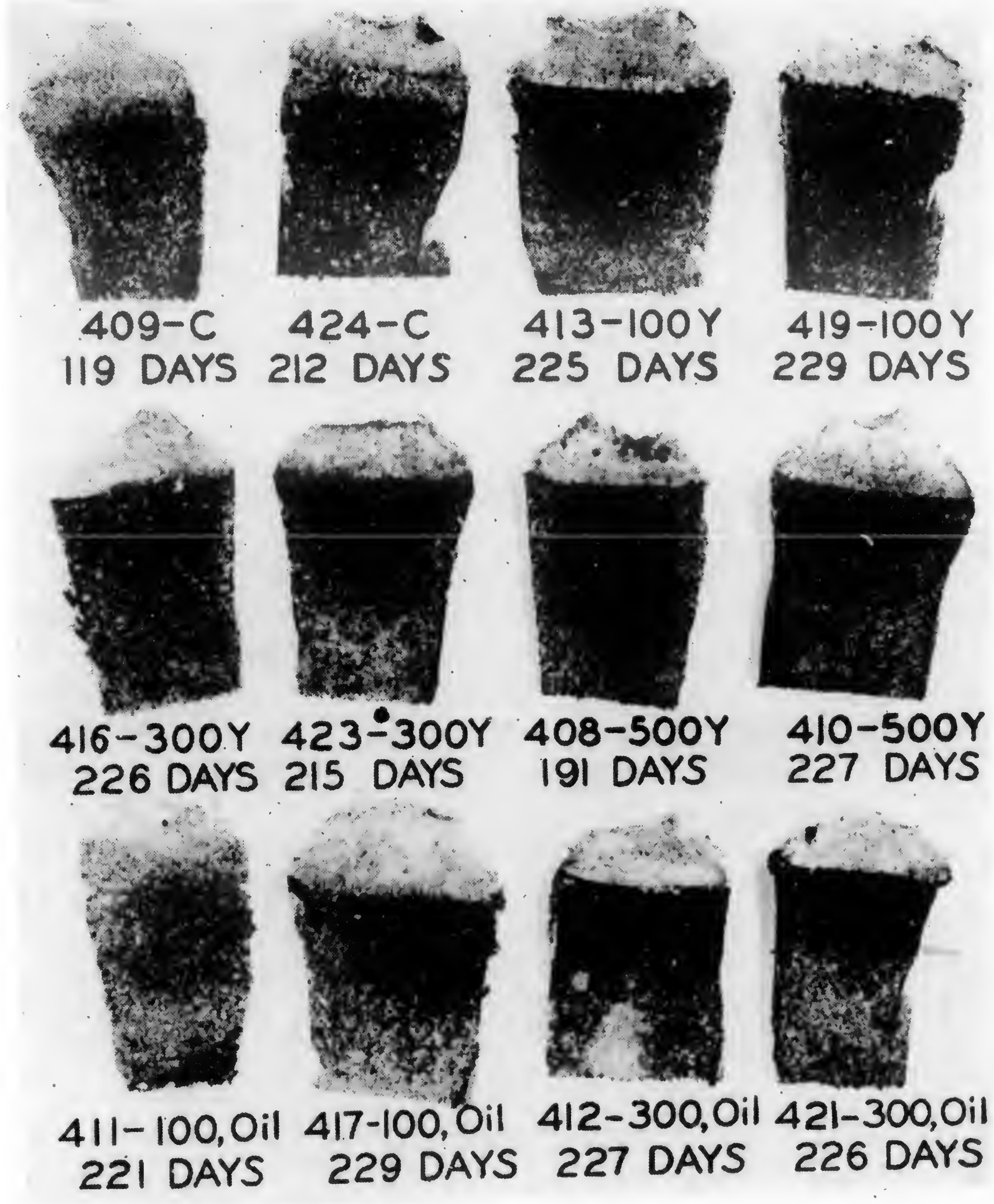

Fig. 8.—Line test showing degree of calcification of distal end of ninth rib. Note especially the poor calcification in control calves 409 and 424.

DISCUSSION OF EXPERIMENTAL RESULTS, 1936-37

The vitamin D potency of the basal diet used in the second year of the experiment was sufficiently low to permit gross development of rickets in the control group. This proved to be true of all the calves in this group with the exception of calf 424. Although this animal exhibited mild rickets, she never developed the usual characteristic outward symptoms in the 212 days she was kept on experiment. The other four control animals developed gross symptoms of rickets. Calf 409 came down first at 142 days of age. She had swollen knee and hock joints and the back was arched. She had considerable difficulty in rising to her feet after lying down. The joints were quite stiff and she walked with a slow, staggering gait. This calf, and the other three controls, stood with her legs bent at the knee joints, and bowing of the legs inward or outward was quite common. The control animals retained normal appetites and ate well except when in a very serious rachitic condition. When they stopped eating, slight improvement usually resulted for a few days. The appetite would soon return and when the normal amount of feed was again consumed, the rachitic condition became more severe than it was before. There was no evidence of avitaminosis deficiency as determined by blood carotene values.

When calf 424 was 195 days of age a large swelling appeared on her throat. A few days later this swelling spread along the angle of the jaw. At the time of slaughter the lump on the jaw was removed to assay it for vitamin D. This special assay was made because this calf did not develop a typical case of rickets as did the other controls. It appeared that the infected jaw might be a source of vitamin D synthesis of sufficient quantity to prevent the onset of gross rickets.

When she was slaughtered small nodules were found on the outer part of the paunch and the intestinal lymph glands were swollen, but of normal color. All of the ribs were badly beaded and the bones were very soft. These observations, together with the inorganic calcium and phosphorus, line test and bone-ash data, reveal that this calf was quite rachitic after all. The bio-assay of the lump taken from the jaw revealed no vitamin D potency.

Calcium and phosphorus intake.—The total intake of elemental calcium and phosphorus supplied to all calves on experiment is shown in table 10. The intake of calcium and phosphorus per 100 pounds live weight is influenced, first of all, by the amounts of these elements in the diet. These are shown in terms of percentage in table 7. It was possible to control the amounts of calcium and phosphorus supplied insofar as it was possible to control the total amount of ration consumed.

The calcium intake, almost without exception, was sufficient to take care of nutritional requirements. The phosphorus intake, however, may be questioned. Henderson and Weakly *(9)* report that 2.5 to 3.9 grams of elemental phosphorus per 100 pounds live weight daily, from birth to 26 months of age, provided normal nutrition.

Vitamin D data.—Table 10 reveals the vitamin D intake for the calves on various levels. Four control animals received 125 to 135 U.S.P. units of vitamin D per 100 pounds live weight per day, this being the average from birth to 7 months of age. Calf 409, which was slaughtered at 191 days of age, received a daily average of 180 U.S.P. units. She was quite small but ate very well. This would account for her vitamin D intake per 100 pounds being greater than that of the other control animals.

The four calves on the 100 U.S.P. unit level, 413, 419, 411 and 417, table 10, had a daily average vitamin D intake ranging from 170 to 210 U.S.P. units per day per 100 pounds live weight. Of this group, calf 411 (on 100 U.S.P. units from cod-liver oil concentrate) had an average intake of 170 units. During the late months of the experiment this animal displayed symptoms of rickets as judged by all criteria used. The remaining three animals were slightly rachitic according to the blood data. However, no gross symptoms of rickets were evident.

On the 300 U.S.P. unit level, calves 416, 423, 412 and 421, no evidence of rickets appeared. The results of all other criteria used on this group agreed very well. The daily average vitamin D intake ranged from 345 to 370 U.S.P. units per 100 pounds live weight, from birth to 7 months of age.

Calves 408 and 410 on 500 U.S.P. units of vitamin D from irradiated dry yeast had a daily intake of 450 to 515 units. Calf 410, which had an average daily intake of 450 U.S.P. units, was quite large at birth. The reason for the lower average daily intake of vitamin D is explainable on the basis of the first feeding period. During this period (42 days) no vitamin D supplement was added to the diet; consequently when the average daily intake was computed (from birth to 225 days of age) it was lower than had been expected. The two calves on the 500 U.S.P. units level appeared normal and healthy throughout the experiment.

Inorganic calcium and phosphorus of the blood.—Normal serum calcium was observed in all animals except the control group and two calves receiving 100 U.S.P. units of vitamin D (calf 419, on irradiated dry yeast, and calf 411, on cod-liver oil concentrate). Although it was expected that the control animals would have low serum calcium, it did not fall as low as in the previous year. The ration used during the first year had a calcium content lower than the phosphorus content, while in the second year the reverse was true. In regard to the two calves on the 100 U.S.P. unit level of vitamin D, it is reasonable to suspect that the low calcium values were probably due to an insufficient supply of vitamin D. The other two calves in this 100 unit group were normal in serum calcium throughout the experimental period.

The inorganic phosphorus values, on the other hand, presented a much different picture. The control animals decreased in inorganic phosphorus, from week to week, until a more or less permanent level was reached. These calves tended to remain at a figure between 3.00 and 4.25 mgm. per 100 c.c. of blood serum, from 120 days of age until the end of the experiment.

The calves on the 100 U.S.P. unit level showed inorganic phosphorus values more or less consistently below normal. There is evidence of insufficient vitamin D, but it should be pointed out that the calves on the 300 and 500 unit levels also tended to show values below normal at times. These inconsistent values may be explainable on the basis of insufficient phosphorus in the diet. Had the phosphorus in the diet been higher it appears possible that rickets in the control animals would have been less severe, or would have come on at a later date. It would appear possible, also, that a higher supply of phosphorus in the ration might have changed the whole picture on the 100 unit level group. This group gave consistent evidence of insufficient vitamin D when judged by all criteria used in the experiment.

The two calves on 500 U.S.P. unit level (irradiated dry yeast) did not show inorganic phosphorus values appreciably better than those on the 300 unit level. This might be interpreted to mean that the calves on the 300 unit level received an ample amount of vitamin D to satisfy the unit requirement, and larger amounts could not change the blood picture. It may be possible that a low daily phosphorus supply was the limiting factor, and normal inorganic phosphorus was impossible on any level of vitamin D supply.

X-ray observations.—From figures 6 and 7, definite conclusions can be drawn concerning x-ray results in the different groups of calves. The animals of the negative control group, whose only source of vitamin D was the basal diet, received 125 to 180 U.S.P. units of vitamin D per calf per 100 pounds live weight per day, table 10. Four of the five animals in this group developed pronounced rickets. The fifth animal, 424, for some unknown reason failed to display clear-cut x-ray evidence of rickets. While the ephiphyseal cartilage of this calf narrowed, the cartilage of the four other controls remained wide, figs. 6 and 7, throughout the experimental period. The x-ray results indicated that calcification took place in calf 424 in a degree about equivalent to that of the calves on the 100 unit level.

The x-ray evidence on the group receiving 100 units of vitamin D in addition to the basal ration, calves 413, 419, 411 and 417, indicated an insufficient amount of vitamin D for full protection against rickets. However, the epiphyseal cartilage in this group did narrow more rapidly than those of the control animals. Calf 411 (on 100 U.S.P. units of vitamin D from cod-liver oil concentrate) did not show as narrow a cartilage as the other animals in this group on the same level. The wide epiphyseal cartilage, in the case of 411, checked very well with the serum calcium, inorganic phosphorus and line test results.

The group on the 300 unit level of vitamin D, calves 416, 423, 412 and 421, produced no x-ray evidence of unsatisfactory calcification. This also holds true for calves 408 and 410 (500 units from irradiated yeast). The gradual narrowing down (calcification) of the epiphyseal cartilage in these calves is clearly evident in the x-ray pictures, fig. 6. The series of pictures taken on the four calves in the 300 unit group, although not shown in this report, were not appreciably different from those of calves 408 and 410 on the 500 unit level.

The curative effect of vitamin D from irradiated dry yeast is shown in figure 7. Calf 414 developed a severe case of rickets. At 182 days of age she was placed on a curative treatment of 500 U.S.P. units from irradiated yeast per day per 100 pounds live weight. The epiphyseal cartilage calcified almost completely in 44 days. Little, if any, change was observed in the epiphyseal cartilage of the control animals, 415 and 429, in this same period. Later, these calves also were put on curative treatment, 415 on 1,500 U.S.P. units and 429 on 3,000 U.S.P. units of vitamin D from irradiated dry yeast. The calcification of the epiphyseal cartilage was found to be almost complete in these calves after 28 days of the curative treatment and all the gross symptoms of rickets had disappeared.

Line test.—Results of the line test, fig. 8, show that the control animals (calves 409 and 424) had the poorest calcification. Calf 408, which received 500 units of vitamin D from irradiated dry yeast, and calf 409, a control, were twins. These two animals were slaughtered at 191 days of age. By comparing these two calves, fig. 8, it can be seen that the calcification of 408 was far superior to that of 409. However, 408 does not show as dense calcification as does 410 which also received 500 units of vitamin D from irradiated dry yeast. It is reasonable to suspect that the calcification of 410 was superior to that of 408, because the former was 36 days older at the time of slaughter.

A comparison of calves 413 and 419 (on 100 units of vitamin D from irradiated yeast) and calves 411 and 417 (on 100 units of vitamin D from cod-liver oil) with the controls, reveals evidence of better calcification because of the irradiated dry yeast or cod-liver oil concentrate in the diet. However, the degree of calcification of the four calves receiving 100 units of vitamin D was not equal to that of the groups on the 300 or 500 U.S.P. unit level. Calf 411 (on 100 units from cod-liver oil) has the poorest calcification of the four in the 100 U.S.P. unit group.

The four calves in the 300 U.S.P. unit group (calves 416 and 423, on irradiated yeast, and 412 and 421, on cod-liver oil) display calcification that is definitely superior to those on the 100 unit levels. The line test on the calves in the 300 unit group indicates a slightly better calcification when vitamin D was supplied by cod-liver oil concentrate as compared with irradiated dry yeast.

Calf 424, a control, displayed inferior calcification in the line test. This result is somewhat in conflict with the x-ray results and the lack of gross symptoms of rickets. With this one exception, the line test results agree very well with other criteria used in this experiment.

Table 11.—Ash content of eighth left rib.

CALF No.	FIRST 10% OF DISTAL END Per Cent	SECOND 10% OF DISTAL END Per Cent	REMAINING 80% Per Cent	TOTAL Per Cent
409	33.90	46.14	50.79	47.50
414*				
415*				
424	39.99	47.93	53.53	50.73
429*				
Control group				
413	47.23	47.27	53.85	52.21
419	42.80	48.57	52.15	50.56
411	32.08	49.00	51.83	48.04
417	42.12	46.79	55.20	52.50
100 units of vitamin D supplement				
416	45.90	51.16	57.14	55.18
423	44.70	49.93	53.56	51.98
412	49.92	54.97	56.50	55.67
421	45.87	49.50	55.85	54.09
300 units of vitamin D supplement				
408	39.33	45.56	52.84	50.66
410	47.04	47.25	53.95	52.29
500 units of vitamin D supplement				

*Not slaughtered, put on curative treatment.

Bone ash.—The bone-ash data, table 11, were more significant than those obtained the previous year. A comparison (total ash) of twin calves, 408 and 409, which were slaughtered at 191 days of age, reveals that the control animal 409 had a lower total ash than 408 which received 500 U.S.P. units of vitamin D from irradiated dry yeast. However, the total ash of 424 is greater than that of 408, but this was probably due to the fact that 424 was 21 days older than 408 at the time of slaughter. It is reasonable to suspect that, had 408 been continued for a longer time, the percentage of bone ash would have been greater.

Calves 413 and 419 (on 100 U.S.P. units from irradiated yeast) and 411 and 417 (on 100 U.S.P. units from cod-liver oil) had a lower total bone-ash percentage than the calves on the higher levels of supplement feeding. Two of these calves, 419 and 411, had a lower total bone ash than did the control, 424. This result agrees with the line test in which 411 showed poor bone calcification. Calves 413 and 417 were considerably higher in bone ash than were the control animals of the same age.

The four animals receiving 300 U.S.P. units of vitamin D (calves 416 and 423 from irradiated yeast, and 421 and 412 from cod-liver oil) were well above the control animals and those on the 100 unit level in total bone-ash percentage. However, calf 423 in this group had a total bone ash comparable to that of the animals on the 100 U.S.P. unit level.

Calf 410, which received 500 U.S.P. units of vitamin D from irradiated yeast, had a rather low percentage of bone ash. This calf grew more rapidly than any of the others. This fast growth might account for the low bone-ash percentage (comparable to the 100 U.S.P. unit level). Had this animal grown more slowly the percentage of total bone ash might have been higher. It appears possible that the inadequate phosphorus in the diet during the last 56 days of feeding may have been the cause of the low bone-ash result on this calf. Furthermore, it is possible that a higher supply of phosphorus to all of the calves on the experiment might have resulted in a more clear-cut bone-ash picture.

In general, the bone-ash data are not out of harmony with other criteria. They do support the contention that the four calves on the 100 U.S.P. unit level, as well as the controls, did not receive sufficient vitamin D to support skeletal calcification. Furthermore, the data indicate that there was an ample amount of vitamin D in the diet of the calves on the 300 U.S.P. unit level to support bone calcification.

SUMMARY

One hundred thirty-five U.S.P. units of vitamin D per day per 100 pounds live weight from birth to 7 months of age were insufficient to prevent rickets in calves. The minimum level of vitamin D for growth, well being and proper calcification of bones of calves from birth to 7 months of age, as found under the conditions of this experiment, is approximately 300 U.S.P. units per day per 100 pounds live weight.

Rickets in calves can be prevented by feeding a vitamin D supplement of irradiated dry yeast or cod-liver oil concentrate. When these are administered to calves, on an equal unit basis, they are equally active in preventing rickets. This can be interpreted to mean that the bovine species can utilize the natural and artificial forms of vitamin D with equal efficiency.

Roentgen photographs of the epiphyseal cartilage at the distal end of the ulna were helpful in making observations on the calcification of bones of living calves.

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A Chemical Study of Mixtures of Lime Sulfur and Lead Arsenate[1]

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Early studies of the lead arsenate and lime sulfur combination, which has become the most common arsenical-fungicidal mixture, indicated that the two components exhibited tendencies to reduce the effectiveness of each other and promote injury to the plant surfaces.

Investigations of the mixture of lead arsenate and lime sulfur both with and without modifying materials have been reported. These investigations have been for the most part attempts to produce spray mixtures without undesirable properties, while maintaining the maximum insecticidal and fungicidal values. Such studies have been based in most cases on practical field experiments, and comparatively little is known of the chemical changes involved in the mixture. Such chemical studies as have been reported have been made with the idea of reducing the soluble arsenic present in the mixture, since the consensus is that this is the principal causative agent in spray injury to plants.

This investigation, dealing as it does only with the chemical changes which occur in the spray solution before application to the host plant, can not answer all the questions concerning the suitability of the spray mixtures studied for practical use. The changes that take place in the film of spray after it has been applied to the plant surface are of major importance, but their study lies outside the field of the present paper. The usefulness of this investigation then lies in the contribution which it may make to the knowledge of the chemistry of the reaction between lead arsenate and lime sulfur under certain specific conditions. It is, however, possible to point out certain implications from the results of this work which may be of considerable practical value.

PREVIOUS STUDIES.—The problem of spray injury by lead arsenate and lime sulfur mixtures has been recognized for many years and has been investigated by many workers, including Fernald & Bourne (1922), Swingle, Morris & Burke (1923), Mogendorff (1925), Ginsburg (1929), Young (1930) and others. The consensus appears to be that the injury is caused by soluble arsenic, and that the amount of soluble arsenic is increased by the addition of lime sulfur. Certain correctives have been suggested to reduce the amount of the injury, and include a variety of substances. Of those suggested, slaked lime and dried skim milk powder alone and in combination have been studied in this work.

The active fungicidal agent present in lime sulfur solutions has been established by Abbott *et al.* (1926), Holland *et al.* (1921) and Tartar (1914) as that form of sulfur present as the calcium polysulfides. Whether the actual mechanism of the toxic action is through its solvent action as suggested by Tartar (1914), through the action of hydrogen sulfide formed or by some other means need not be discussed here.

THE PRESENT PROBLEM.—It appears then that, aside from those compounds such as the thiosulfates, sulfates and others the determination of which under various conditions gives information as to the mechanism of the chemical reactions between the components of the mixture, the two most important constituents of any spray mixture having as its basis lime sulfur and lead arsenate are the soluble arsenic and the polysulfide sulfur. The ideal condition would be to keep the former as low and the latter at as high a level as possible in the mixture.

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[2] Submitted by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agricultural and Biological Chemistry in the Graduate School of the Pennsylvania State College, June, 1937.

[3] Early publication of this paper has been secured by payment of the printing charge.

The following experiments have been planned to indicate the chemical changes which take place in lime sulfur solutions when used in combination with lead arsenate alone and with recognized adjuncts: lime and dried skim milk powder. These studies included not only the determination of soluble arsenic and soluble calcium but also a quantitative investigation of the soluble sulfur compounds present in the mixtures, of which the polysulfide sulfur has been considered to be the active fungicidal agent. Studies were also made on the effect of various physical factors such as temperature, time of agitation, etc., on the decomposition of lime sulfur alone and in mixtures.

In preparing the experimental mixtures, conditions used in commercial orchard practice were followed as far as possible. The period of agitation of the mixtures was limited to one hour as the maximum time that a spray solution remains in the spray tank under constant stirring.

MATERIALS USED.—The following materials were used in the experiment.

Liquid Lime Sulfur.—A commercial grade, concentrated lime sulfur solution was used having a specific gravity of 1.2762 and a Baumé reading of 31.62 degrees. Its total sulfur content was 24.05 per cent.

Acid Lead Arsenate.—A chemically pure powder purchased from the Baker Chemical Company. On analysis it was found to contain:

	Per Cent
Total PbO	65.70
Total Arsenic Oxide	31.98
Water Soluble Arsenic as As_2O_5	0.53
Moisture	0.50

Calcium Hydroxide.—A chemically pure grade, heated before use to insure removal of carbonates.

Skim Milk Powder.—A commercial product prepared for spray purposes, yellow in color and containing by analysis 69.23 per cent protein. It was separated by screening before use and a single size used for all of the experiments. This passed through a 60- but not 80-mesh screen.

PREPARATION OF EXPERIMENTAL MIXTURES.—After mixing the several ingredients, whatever the number chosen, the final volume was brought to one liter. The manipulation was as follows in all cases: Approximately 900 ml. of water were placed in a 2-liter beaker fitted with a ground glass top and an electric stirrer. Unless otherwise noted, distilled water was used. The solid ingredients were added to the water and, while the mixture was stirred, lime sulfur concentrate was added in quantity sufficient for the dilution desired. Water was then added to make the final volume of one liter. The mixture was stirred for the period desired at a constant rate of 200 revolutions per minute. The mixture was filtered immediately through a Gooch crucible fitted with a hard filter paper base.

The filtrate was sampled (without washing the residue) for total sulfur, sulfide sulfur, thiosulfate sulfur, calcium and soluble arsenic by the methods indicated below.

The residue was washed with a very small quantity of cold distilled water until the washings were free from the yellow color of lime sulfur solution. The residues were dried for two hours at 60 degrees and retained in vacuo over sulfuric acid until constant weight was reached.

If the filtrate was to be stored for later analysis, the container was filled to exclude air, capped and waxed, and stored in a dark place.

METHODS OF ANALYSIS.—The methods used for determining total sulfur, sulfide sulfur, thiosulfate sulfur and water soluble arsenic (hydrazine sulphate and sodium bromide distillation) were those given in the Official Methods of the Association of Official Agricultural Chemists (1935). Calcium was determined by the McCrudden (1911) method as modified by Halverson & Shulz (1920). Hydrogen sulfide was estimated by placing the closed system under a slight vacuum and drawing the evolved gas through a solution of cadmium chloride as suggested by Scott (1925).

EFFECT OF DILUTION ON THE CONSTITUENTS OF LIME SULFUR CONCENTRATE.—The concentrated lime sulfur solution, specific gravity 1.2762 and Baumé 31.62 degrees, was diluted to various degrees between 1–10 and 1–200 and analyzed as a preliminary experiment. The analyses indicated that within the dilution range studied and under comparable conditions there was no appreciable change in the percentage of the constituents of the solutions. The oxidation change appeared to proceed slowly during the first hour under these conditions and the differences in the percentage of the active sulfide in the diluted solutions were considered negligible from a practical standpoint.

Table 1.—Effect of temperature on the constituents of diluted lime sulfur.

Quarts Concentrate Per 100 Gallons	4		8		12		16	
Dilution Radio of Lime Sulfur	1–100		1–50		1–33.3		1–25	
Temperature in Degrees C.	23	32	23	32	23	32	23	32
Total Sulfur (g. per 100 gal.)	1118.500	1101.600	2244.200	2183.200	3370.800	3321.500	4487.600	4398.900
Sulfide Sulfur (g. per 100 gal.)	1042.900	1015.700	2106.800	2036.600	3161.400	3075.700	4209.200	4059.600
Thiosulfate Sulfur (g. per 100 gal.)	67.200	74.100	124.800	142.800	186.900	215.000	248.800	302.800
Sulfate Sulfur (g. per 100 gal.)	8.400	12.000	15.200	23.800	22.500	30.800	29.600	36.500
Calcium (g. per 100 gal.)	326.500	329.400	650.800	651.600	975.900	973.900	1299.600	1301.600
Sulfide Sulfur (per cent)	93.500	92.200	93.870	92.280	93.780	92.600	93.790	92.280
Mol Ratio S/Ca (as sulfide)	4.757	4.734	4.755	4.779	4.755	4.798	4.752	4.767

EFFECT OF TEMPERATURE VARIATION.—The concentrated lime sulfur was diluted in proportions of 1–25, 1–33.3, 1–50 and 1–100. These mixtures were prepared according to the standard procedure and were stirred at a constant temperature of 32 degrees C. Table 1 is a comparison of the analyses of these solutions with similar mixtures prepared at 23 degrees C.

A rise in temperature increased the rate of decomposition of the mixtures, resulting in a decreased percentage of sulfide sulfur in the solution. There was also an increase in thiosulfate sulfur in compensation to the loss of sulfur as the sulfide. Little change appeared in secondary products resulting from the decomposition. The actual percentage of the sulfide sulfur in the solution decreased approximately 1½ per cent.

The increase in the ratio of sulfur to calcium in the sulfide form in the solutions, as the temperature was increased, indicated a rise in the concentration of the polysulfide in solution as compared to the total sulfur in solution. Since the total calcium in the solution remained at the same level within limits of experimental error, and the sulfur as the sulfate increased only to a slight degree, it appeared that the change due to increased temperature resulted from an increased hydrolysis of the mixture. Sulfur was lost from the solution, apparently as hydrogen sulfide, since no free sulfur was precipitated from the solution. The free sulfur, which is normally precipitated from lime sulfur solution on oxidation and formation of the thiosulfate and sulfate sulfur, was then redissolved in the solution, where it retained the sulfide concentration at its high level.

EFFECT OF TIME.—Concentrated lime sulfur diluted 1–50 was prepared and stirred according to the standard procedure at a constant temperature of 23 degrees C. for periods ranging between 1 and 45 hours. Analyses were made of each of the solutions in order to determine the extent of the decomposition process.

Under the conditions of the experiment, the sulfide sulfur of the solution was almost entirely removed in 45 hours, table 2. The loss of sulfur as the sulfide was proportional to the increase in the concentration of the thiosulfate sulfur and sulfate sulfur in solution. The decomposition progressed slowly in the initial stages but appeared to increase rapidly in the later stages of agitation.

Table 2.—Effect of time of stirring on the constituents of lime sulfur diluted 1–50 (23 degrees C.).

HOURS STIRRED	1	5	10	20	30	40	45
Total Sulfur (g. per 100 gal.)	2244.200	1912.000	1630.400	1318.100	1067.600	789.600	758.300
Sulfide Sulfur (g. per 100 gal.)	2106.800	1702.600	1347.100	916.600	523.600	189.900	11.100
Thiosulfate Sulfur (g. per 100 gal.)	124.800	154.300	201.500	301.100	377.200	443.000	607.600
Sulfate Sulfur (g. per 100 gal.)	15.200	55.100	81.800	100.400	166.800	156.700	139.600
Calcium (g. per 100 gal.)	650.800	652.600	635.400	623.800	645.800	647.700	652.300
H_2S Evolved (g. per 100 gal.)	2.000	10.200	12.400	12.600	11.200	11.900	12.100
Sulfide Sulfur (per cent)	93.870	98.040	82.630	69.540	49.040	24.050	1.460
Mol Ratio S/Ca (as sulfide)	4.755	4.437	3.695	3.411	2.945	1.939	0.141

Free sulfur was precipitated from the solution during the first five hours and continued to be precipitated throughout the 45 hour period. From the analyses it appears that during the period approximately one-third of the total sulfur present in the solution was precipitated as the free sulfur. Hydrogen sulfide evolved from the mixture was small, indicating that the decomposition was primarily due to oxidation. As the solution became more dilute with respect to the concentration of the polysulfides, the oxidative process was increased.

Table 3.—Effect, on the constituents of the solutions, of the addition of three pounds of lead arsenate to diluted lime sulfur solutions.

Quarts Concentrate Per 100 Gallons	2	4	8	12	16	20	40
Dilution Ratio of Lime Sulfur	1–200	1–100	1–50	1–33.3	1–25	1–20	1–10
Total Sulfur (g. per 100 gal.)	87.300	605.400	1765.300	2902.700	4016.700	5145.600	10577.500
Sulfide Sulfur (g. per 100 gal.)	13.100	470.200	1576.400	2632.300	3706.600	4773.600	9806.200
Thiosulfate Sulfur (g. per 100 gal.)	67.800	108.200	145.400	197.800	250.400	298.900	615.700
Sulfate Sulfur (g. per 100 gal.)	6.400	27.000	43.500	52.600	59.700	73.100	156.600
Calcium (g. per 100 gal.)	66.700	223.700	543.200	851.600	1163.600	1483.900	3064.400
Soluble As_2O_5 (g. per 100 gal.)	19.900	17.100	12.100	9.000	7.800	6.600	6.200
Residue (g. per 100 gal.)	1701.700	1964.400	1978.800	1992.500	1998.800	1819.900	1662.900
Sulfide Sulfur (per cent)	14.890	77.660	89.290	90.680	92.280	92.770	92.780
Mol Ratio S/Ca (as sulfide)	1.010	4.855	4.952	4.969	4.967	4.947	4.935

The ratio of sulfur to calcium in the sulfide form in the solution decreased at a regular rate, indicating that sulfur as free sulfur was the only oxidative product lost from solution. The loss of calcium during the middle stages of the decomposition and its recovery towards the end of the stirring period may be due to a precipitation of oxidation products later redissolved in solution as the thiosulfate and other oxidation products increase. These results agree with the analyses reported by Fernald (1907).

Effect of Addition of Acid Lead Arsenate.—Workers agree that the addition of acid lead arsenate to lime sulfur solutions results in a loss of sulfide sulfur from the solution and an increase in thiosulfate and sulfate sulfur. In addition a residue is formed and precipitated. There is further, reduction in soluble sulfur due to the precipitation of lead sulfide and the formation of an arsenate of calcium. The loss of sulfur due to the formation of lead sulfide has been regarded as the cause of the removal of calcium from the solution. It has been claimed by Goodwin & Martin (1925) and by Ruth (1913) that thioarsenates are formed in the process.

Dilutions of the concentrated lime sulfur solution were prepared which ranged between 1–10 and 1–200. Lead arsenate was added to the dilute lime sulfur in amounts equivalent to 3 pounds in 100 gallons of solution. Each member of the series was treated according to the standard procedure at a constant temperature of 23 degrees C. The results of the analyses, table 3, were calculated on the basis of grams in 100 gallons of mixture.

The relative proportions of the constituents in the diluted mixtures did not vary directly with dilution as in the case of lime sulfur solutions prepared in a similar manner. The actual percentage of sulfide sulfur present in the solution was practically constant in dilutions more concentrated than 1–50. In the very dilute solutions there was an increase in the amount of thiosulfate and other soluble oxidation products which suggests that the loss of sulfur as the sulfide was not due in these cases to the direct addition of lead arsenate but rather to an increased oxidation of the mixture in the presence of lead arsenate.

The addition of lead arsenate measurably reduced the sulfide content of the more dilute mixtures. However, in solutions of higher concentration, the percentage change of the sulfide sulfur was small. For example, in the 1–200 dilution, the percentage of sulfide sulfur was reduced from 92.25 to 14.89 per cent. For the 1–10 mixture, however, the loss was only from 93.82 per cent to 92.78 per cent.

The sulfide concentration in the 1–10 dilution of the mixture, calculated upon the basis of the total sulfur added to the solution, shows a greater reduction of sulfide from the solution. These values show a reduction of from 93.82 per cent to 88.84 per cent.

The relative increase in the calcium polysulfide concentration is illustrated in the sulfur to calcium ratio in the sulfide form. The increase of the ratio from an average value of 4.755 in the case of lime sulfur alone to a value of 4.954 indicates that the proportion of the pentasulfide in the mixture has increased due to the addition of the lead arsenate to solutions more concentrated than 1–50.

The increase in the ratio may be traced to the decomposition of lead arsenate by hydrogen sulfide in the solution. As the lead is precipitated from the lead arsenate, calcium is removed from the calcium polysulfide by arsenic acid produced and precipitated from the solution as an arsenate of calcium. Removal of calcium from the solution in a constant quantity and in a greater proportion than the loss of sulfur either as the sulfide of lead or as free sulfur should increase the molecular ratio between them.

According to Thatcher & Streeter (1924) and Young (1930), the extent of hydrolysis of the polysulfides resulting in the formation of hydrogen sulfide in lime sulfur solution appears to be dependent upon the dilution of the solution. The increased formation of soluble arsenic in the solutions from lime sulfur and lead arsenate mixtures as the dilution was increased would appear as a result of an increased formation of hydrogen sulfide and also as an aid to the further decomposition of the polysulfide in the solution.

Effect of Type of Water.—An experiment was performed using distilled water, tap water and water freed from carbon dioxide as the diluting agents. The lime sulfur solutions were prepared according to the standard procedure in which a dilution of 1–50 of the lime sulfur was used. Lead arsenate was added in the proportion of three pounds in 100 gallons. The results indicated that there was little difference in the primary sulfur containing compounds in the mixture when the different waters were used. Comparative results may be obtained using any of these waters, although the results could be more readily duplicated if distilled water were used.

Effect of Time and Temperature—In a series mixtures of lime sulfur and lead arsenate the lime sulfur was diluted at the rate of 1–50 and lead arsenate added in the proportion of 3 pounds in 100 gallons. The mixtures were prepared according to the general procedure. Each mixture was stirred for a period and the solution analyzed. Successive mixtures were prepared and sampled after longer periods until an analysis of the solutions indicated a disappearance of the sulfide sulfur in the solution. Constant temperatures in the two series of experiments were maintained at 23 degrees C. and 32 degrees C. The results are shown in figs. 1 and 2.

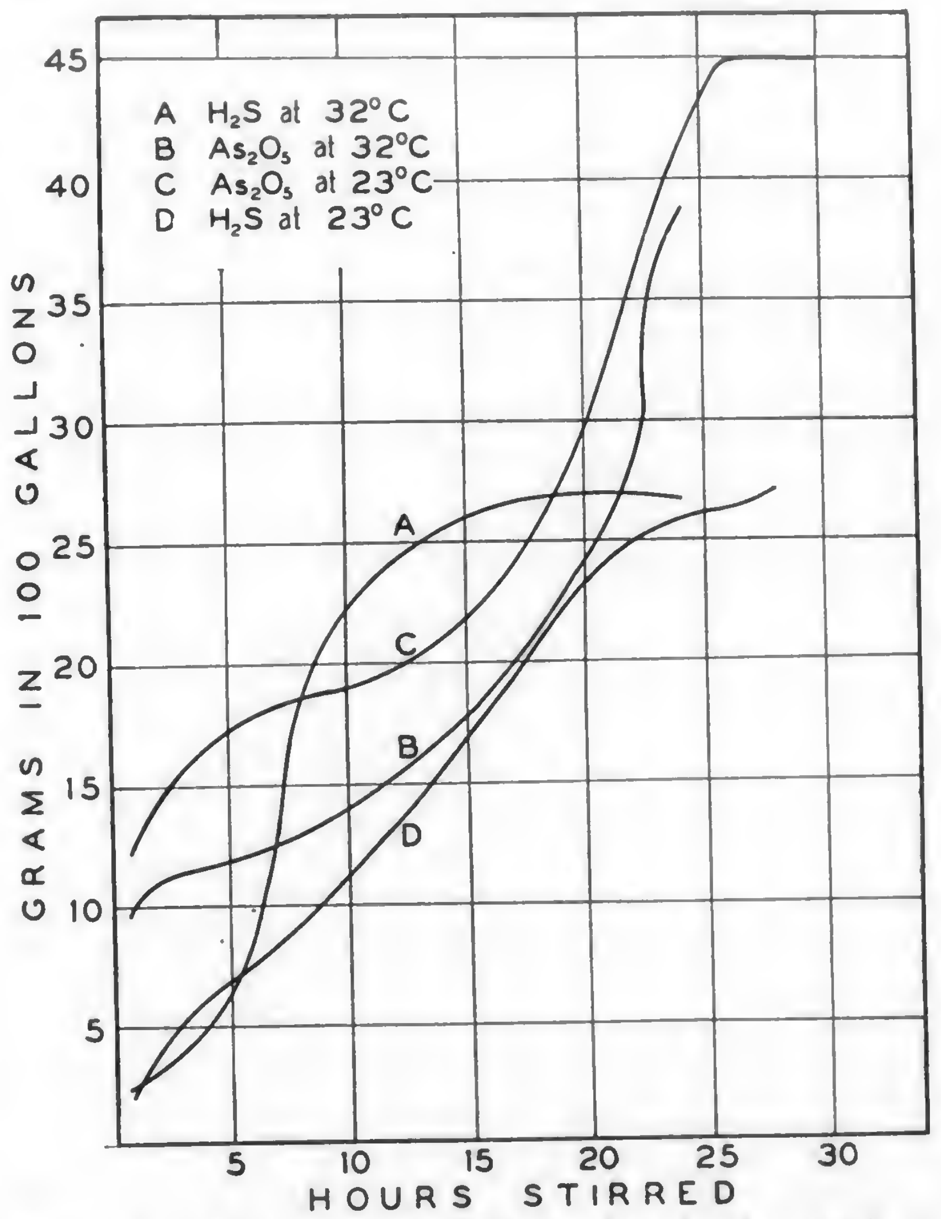

Fig. 1.—Hydrogen sulfide and soluble As_2O_5 in relation to time of stirring and temperature.

The decomposition of the polysulfides in the solution when in contact with lead arsenate proceeded rapidly during the first hour of contact and between the fourth and tenth hours, after which the decomposition appeared to be constant.

A decrease in the sulfide sulfur content was reflected to some extent by an increase in the thiosulfate sulfur soluble in the solution. However, the greatest loss to the solution was the free or precipitated sulfur passing into the residue. Partial analyses of the residues showed that the proportion of free sulfur precipitated increased to approximately the twenty-second hour, beyond which there was no appreciable increase. Thereafter the loss of sulfur from the sulfide may be accounted for by an increase in the thiosulfate sulfur in solution.

The proportion of sulfur remaining, calculated as the sulfate, showed a marked increase between the sixteenth and twenty-fourth hours, where the precipitation of sulfur as free sulfur ceased and the increase in thiosulfate sulfur in the solution was at its greatest rate.

Complete decomposition of the sulfide was reached in 30 hours in the mixture stirred at 23 degrees C. The sulfide sulfur in the mixtures stirred at a temperature of 32 degrees C. was completely removed in 23 hours.

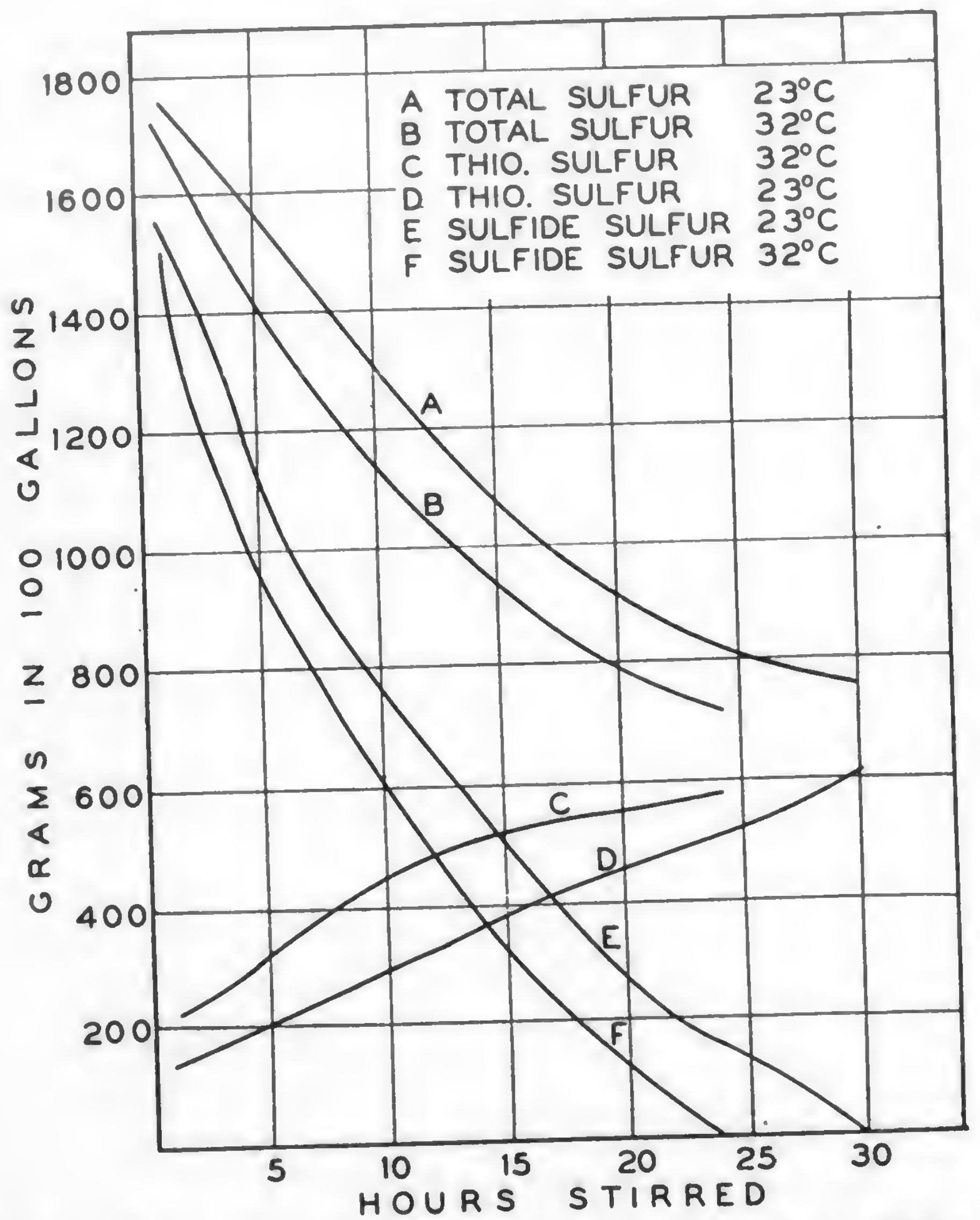

Fig. 2.—Total sulfur, thiosulfate sulfur and sulfide sulfur in relation to time of stirring and temperature.

The principal effect of a change in temperature was an alteration of the rate of decomposition process as shown in fig. 1. The curves are comparative over the entire range of the stirring period and progress in a similar manner independent of the temperature.

The water soluble arsenic in the mixtures stirred at 32 degrees C. was less than the amount found in the solutions stirred at 23 degrees C. Fig. 2 shows the relation between the temperatures and the presence of soluble arsenic and hydrogen sulfide in the solutions. At the higher temperature the hydrogen sulfide was more quickly removed from the solution. It thus prevented the reaction between it and lead arsenate from forming arsenic acid as the end product of the reaction.

The soluble arsenic in the mixture of lime sulfur and lead arsenate stirred at 23 degrees C. increased at a constant rate to the sixteenth hour, when there was an abrupt rise in the proportion soluble in solution. This increase corresponded to the analyses for hydrogen sulfide evolved from the solution, which increased to approximately the sixteenth hour and thereafter was nearly constant. Similar curves are shown for analyses of the soluble arsenic and hydrogen sulfide on the mixture stirred at 32 degrees C. In this case, however, the change occurred at approximately the twenty-second hour of agitation.

Effect of Added Lime.—The changes that occur in the composition of a spray of mixed lime sulfur and lead arsenate as shown by the analyses of solution in the previous experiments, particularly the marked reduction in the amount of polysulfide sulfur present in solution, emphasize the need for the addition of some material which will retard or completely overcome the tendency for chemical reaction between the members of the mixture.

The use of slaked lime to prevent the formation of soluble arsenic was proposed by Robinson (1919), who had previously shown that its use with acid calcium arsenate lowered the amount of soluble arsenic in the solution. He advised the addition of 10 pounds of lime to 100 gallons of lime sulfur solution previous to the addition of lead arsenate and indicated that the polysulfide content under these conditions decreased very little.

In order to determine the effect of lime on the combination of lead arsenate and lime sulfur, five dilutions were prepared which ranged between 1–20 and 1–100. These were treated according to the standard procedure with lime added in the proportion of 1 pound in 100 gallons of mixture. The analyses of these mixtures are shown in table 4 and are calculated in terms of grams in 100 gallons of the mixture.

From table 4 it appears that through the addition of 1 pound of slaked lime per 100 gallons of spray mixture the quantity of sulfur (both total and as the sulfide) in solution was greater than when no lime was present in a similar mixture of lead arsenate and lime sulfur. The difference in the per cent sulfide sulfur appeared as an increase in the total quantity of thiosulfate and sulfate sulfur in solution. These increased in the proportion of twofold and tenfold, respectively.

Table 4.—Variation in the constituents of the solution when lime was added to diluted mixtures of lime sulfur and lead arsenate.

Proportion	4–3–1–0*	8–3–1–0	12–3–1–0	16–3–1–0	20–3–1–0
Dilution Ratio of Lime Sulfur	1–100	1–50	1–33.3	1–25	1–20
Total Sulfur (g. per 100 gal.)	1115.500	2245.800	3370.000	4491.100	5606.200
Sulfide Sulfur (g. per 100 gal.)	778.100	1795.700	2802.800	3817.000	4819.300
Thiosulfate Sulfur (g. per 100 gal.)	202.000	305.400	419.200	506.700	606.500
Sulfate Sulfur (g. per 100 gal.)	135.400	144.700	148.000	167.400	180.400
Calcium (g. per 100 gal.)	492.500	827.100	1159.500	1493.000	1821.100
Soluble As_2O_5 (g. per 100 gal.)	8.400	6.100	5.500	4.800	4.100
Residue (g. per 100 gal.)	1452.300	1467.200	1458.700	1463.600	1461.700
Sulfide Sulfur (per cent)	69.750	79.950	84.060	84.900	85.780
Mol Ratio S/Ca (as sulfide)	4.936	4.927	4.951	4.933	4.951

* The first figure represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked lime; the fourth, pounds of dried skim milk; all figures are on the basis of 100 gallons of spray mixture.

Excess calcium reduced the soluble arsenic in the mixtures to a minimum of 4.1 grams per 100 gallons as compared to a minimum of 6.2 grams in mixtures of lime sulfur and lead arsenate. In water solution, pure lead arsenate was soluble to the extent of 6.8 grams per 100 gallons.

The major effect of the addition of one pound of lime to diluted mixtures of lime sulfur and lead arsenate was a retention of sulfur in solution. Soluble arsenic was reduced to a minimum, and the ratio of sulfide sulfur to sulfide calcium retained at its high level. The difference between the weights of these precipitates and those of a similar series where lime sulfur and lead arsenate were combined (table 3) was 506.8 grams per 100 gallons. This value closely approximated the quantity of lead sulfide (507.3 grams) which was found on partial analysis of the residues from similar mixtures of lime sulfur and lead arsenate.

In order to determine the effect of the addition of larger quantities of lime to the lime sulfur and lead arsenate mixtures, lime sulfur was diluted to 1–50 and lead arsenate added at the rate of 3 pounds to 100 gallons. Lime was added to these in proportion varying between 8 ounces and 9 pounds per 100 gallons of solution. The values for the components of the solution, as shown in table 5, are calculated in terms of grams in 100 gallons of mixture.

The precipitation of sulfur resulting from the addition of lead arsenate was retarded when lime was added in any quantity between 8 ounces and 9 pounds.

The principal effect of the addition of lime was to increase the proportion of soluble oxidation products in the solution. In the mixtures analyzed which contained between 8 ounces and 3 pounds of lime, the state of the polysulfides in the solution appeared to be constant as indicated by the sulfur to calcium ratio. When 6 or 9 pounds of lime were added, the ratio indicated that the polysulfides in the solution were made up primarily of the tetrasulfide rather than the pentasulfide.

As the proportion of lime added to the solution was increased between the limits of 8 ounces and 9 pounds, an increased

Table 5.—Effect on the constituents of the solution when lime was added to mixtures of lime sulfur and lead arsenate diluted 1–50.

Proportion	8–0–0–0*	8–3–0–0	8–3–½–0	8–3–1–0	8–3–2–0	8–3–3–0	8–3–6–0	8–3–9–0
Total Sulfur (g. per 100 gal.)	2244.200	1765.300	2241.400	2245.800	2248.400	2252.000	2247.200	2244.500
Sulfide Sulfur (g. per 100 gal.)	2106.800	1576.400	1978.900	1795.700	1740.600	1706.500	1791.000	2038.400
Thiosulfate Sulfur (g. per 100 gal.)	124.800	145.400	181.700	305.400	310.200	373.400	344.600	150.400
Sulfate Sulfur (g. per 100 gal.)	15.200	43.500	80.800	144.700	197.600	172.100	111.600	55.700
Calcium (g. per 100 gal.)	650.800	543.200	714.800	827.100	891.600	904.600	919.400	946.400
Soluble As_2O_5 (g. per 100 gal.)		12.100	6.400	6.100	5.400	3.900	3.300	3.200
Residue (g. per 100 gal.)		1978.800	1417.100	1467.200	1802.500	2130.100	3325.200	4584.100
Sulfide Sulfur (per cent)	93.870	89.290	88.280	79.950	77.430	75.770	79.630	91.260
Mol Ratio S/Ca (as sulfide)	4.755	4.952	4.942	4.927	4.826	4.676	3.965	3.253

* The first figure represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked lime; the fourth, pounds of dried skim milk; all figures are on the basis of 100 gallons of spray mixture.

Table 6.—Effect on the constituents of the solution when dried skim milk was added to mixtures of diluted lime sulfur and lead arsenate.

PROPORTION	4–3–0–1*	8–3–0–1	12–3–0–1	16–3–0–1	20–3–0–1
DILUTION RATIO OF LIME SULFUR	1–100	1–50	1–33.3	1–25	1–20
Total Sulfur (g. per 100 gal.)	712.600	1828.200	2938.600	4063.700	5196.400
Sulfide Sulfur (g. per 100 gal.)	639.500	1654.400	2680.600	3732.900	4772.800
Thiosulfate Sulfur (g. per 100 gal.)	69.300	143.600	232.600	308.800	403.500
Sulfate Sulfur (g. per 100 gal.)	22.700	30.200	25.400	22.000	20.100
Calcium (g. per 100 gal.)	245.400	552.600	868.400	1186.300	1502.700
Soluble As_2O_5 (g. per 100 gal.)	16.500	15.300	14.800	15.000	14.700
Residue (g. per 100 gal.)	1822.500	1864.100	1842.700	1872.700	1831.600
Sulfide Sulfur (per cent)	89.740	90.490	91.220	91.860	92.040
Mol Ratio S/Ca (as sulfide)	4.883	4.864	4.848	4.831	4.867

* The first figure represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked lime; the fourth, pounds of dried skim milk; all figures are on the basis of 100 gallons of spray mixture.

residue resulted. Hydrogen sulfide was not evolved in detectable quantities from any of the mixtures to which lime had been added. Arsenic rendered soluble in the solution was reduced as the quantity of lime was increased.

From the standpoint of the chemical stability of the solution under the conditions of the experiments, the addition of 8 ounces of lime was sufficient to prevent a reaction resulting in the formation of lead sulfide in the residue. The addition of lime did not prevent the decomposition of the sulfides in the solution in a manner proportional to the amount added. The use of lime in quantities of 8 ounces, 1, 2 and 3 pounds aided in the decomposition of the sulfides as the quantity was increased. However, the addition of lime in larger quantities of 6 and 9 pounds reversed the reaction and returned the sulfide content of the solution to a level approximating that of lime sulfur solution at a similar concentration.

Triple mixtures containing lime in quantities of 8 ounces, 1, 2 and 3 pounds contained quantities of polysulfide sulfur nearly as great as lime sulfur solution alone. No apparent loss was found in the total sulfur which was added to the mixture in the form of lime sulfur solution. The addition of lime in all cases prevented a decomposition similar to that shown in the analysis of the solution from lime sulfur and lead arsenate mixtures.

EFFECT OF ADDED DRY SKIM MILK POWDER.—The use of casein in spray mixtures has been advocated by many workers. Various preparations have been used, including liquid whole, condensed or skimmed milk, dried milk powders, and combinations of milk preparations with lime, loosely termed "casein-lime" or calcium caseinate. It apparently has been the general opinion that these materials act in two ways: through their colloidal properties as wetting, spreading and sticking agents, or through means not so clearly understood as preventives of the formation of undesirable decomposition products.

While it is beyond the scope of this paper to investigate the possible action of casein on the physical properties of spray mixtures, the effect of dried skim milk powder on the chemical composition of mixtures containing lime sulfur and lead arsenate was studied. Mixtures of lime sulfur, diluted within the range of 1–10 and 1–100, were prepared according to the standard procedure. Lead arsenate was added at the rate of 3 pounds and dried skim milk at the rate of 1 pound in 100 gallons of solution. The results are shown in table 6.

Table 7.—Variation of the constituents of solution upon the addition of dried skim milk to mixtures of lime sulfur and lead arsenate diluted 1–50.

PROPORTION	8–0–0–0*	8–3–0–0	8–3–0–½	8–3–0–1	8–3–0–2	8–3–0–3
Total Sulfur (g. per 100 gal.)	2244.200	1765.300	1795.200	1828.200	1835.100	1854.300
Sulfide Sulfur (g. per 100 gal.)	2106.800	1576.400	1627.800	1654.400	1658.500	1671.100
Thiosulfate Sulfur (g. per 100 gal.)	124.800	145.400	135.300	143.600	151.300	166.000
Sulfate Sulfur (g. per 100 gal.)	15.200	43.500	32.100	30.200	25.300	17.200
Calcium (g. per 100 gal.)	650.800	543.200	545.400	552.600	548.600	549.900
Soluble As_2O_5 (g. per 100 gal.)		12.100	14.500	15.300	16.200	16.400
Residue (g. per 100 gal.)		1978.800	1764.600	1864.100	1963.600	2120.300
Sulfide Sulfur (per cent)	93.870	89.290	90.660	90.490	90.380	90.120
Mol Ratio S/Ca (as sulfide)	4.755	4.952	4.835	4.864	4.906	4.918

* The first figure represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked ime; the fourth, pounds of dried skim milk; all figures are on the basis of 100 gallons of spray mixture.

Table 8.—Effects of the addition of lime and dried skim milk upon the constituents of mixtures of lime sulfur and lead arsenate diluted 1–50.

PROPORTION	8–0–0–0*	8–3–0–0	8–3–½–½	8–3–½–1	8–3–½–2	8–3–½–3	8–3–1–½	8–3–1–1	8–3–1–2
Total Sulfur (g. per 100 gal.)	2244.200	1765.300	2243.600	2240.600	2243.700	2245.200	2247.600	2245.300	2245.400
Sulfide Sulfur (g. per 100 gal.)	2106.800	1576.400	1958.200	1847.600	1798.600	1710.600	1863.700	1950.200	1904.600
Thiosulfate Sulfur (g. per 100 gal.)	124.800	145.400	127.900	255.300	315.400	409.400	231.600	130.500	200.400
Sulfate Sulfur (g. per 100 gal.)	15.200	43.500	155.500	137.700	129.700	125.200	152.300	164.600	140.400
Calcium (g. per 100 gal.)	650.800	543.200	804.800	812.700	833.600	852.600	838.700	836.600	852.700
Soluble As_2O_5 (g. per 100 gal.)		12.100	6.400	7.300	9.900	12.600	6.000	6.300	6.600
Residue (g. per 100 gal.)		1978.800	1331.500	1454.700	1468.600	1536.800	1512.800	1640.200	1682.900
Sulfide Sulfur (per cent)	93.870	89.290	87.280	82.460	80.160	76.630	82.920	86.850	84.820
Mol Ratio S/Ca (as sulfide)	4.755	4.952	4.612	4.700	4.744	4.857	4.625	4.421	4.312
PROPORTION	8–3–1–3	8–3–2–½	8–3–2–1	8–3–2–2	8–3–2–3	8–3–3–½	8–3–3–1	8–3–3–2	8–3–3–3
Total Sulfur (g. per 100 gal.)	2246.200	2239.700	2241.800	2246.700	2240.600	2246.500	2247.600	2242.200	2245.900
Sulfide Sulfur (g. per 100 gal.)	1834.900	1803.500	1842.100	1946.300	1872.300	1765.600	1801.700	1920.400	1939.200
Thiosulfate Sulfur (g. per 100 gal.)	276.000	212.800	235.100	136.600	242.700	260.200	197.200	164.600	142.600
Sulfate Sulfur (g. per 100 gal.)	135.300	223.400	184.600	163.800	125.600	220.700	248.700	157.200	164.100
Calcium (g. per 100 gal.)	864.800	884.700	896.800	902.500	917.600	918.600	923.300	925.700	928.700
Soluble As_2O_5 (g. per 100 gal.)	7.800	5.700	7.400	5.300	8.400	4.100	4.200	4.600	4.500
Residue (g. per 100 gal.)	1784.700	1998.600	2092.700	2114.600	2146.800	2226.500	2312.100	2484.700	2541.700
Sulfide Sulfur (per cent)	81.760	80.520	82.610	86.630	83.560	78.430	80.110	85.340	86.140
Mol Ratio S/Ca (as sulfide)	4.384	4.672	4.453	3.972	3.842	4.598	4.604	3.832	3.790

* The first figure represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked lime; the fourth, pounds of dried skim milk; all figures are on the basis of 100 gallons of spray mixture.

The primary influence of the addition of dried skim milk was a diminution of the decomposition of the solution. The sulfide concentration was greater than that found in similar mixtures containing lime sulfur and lead arsenate. The protection from decomposition of the sulfide sulfur in the solution was not as great as that found when lime in the quantities used was added to like dilutions of the mixture.

Oxidation of the lime sulfur solution was retarded, as shown by the reduced quantities of thiosulfate and sulfur as the sulfate in solution.

A series of mixtures was prepared containing lime sulfur, diluted 1–50, lead arsenate at the rate of 3 pounds and dried skim milk in quantities varying between 8 ounces and 3 pounds in 100 gallons of solution. The results are shown in table 7.

The analyses of the solutions showed results similar to those of the previous experiment. Sulfide sulfur was retained above the value calculated to be present in mixtures of lime sulfur and lead arsenate. The addition of dried skim milk slightly increased the quantity of total sulfur retained in the solution while the sulfide concentration appeared to vary only slightly over the entire range of addition of the dried skim milk.

Comparisons between the analyses of solutions from mixtures of lead arsenate and lime sulfur containing lime and those containing dried skim milk in similar quantities indicate that lime was of greater benefit in the retention of total and of sulfide sulfur in the solution.

Soluble arsenic in the solutions containing dried skim milk was increased as the proportion of dried skim milk was increased. This has been explained by Goodwin & Martin (1925) and by Thatcher & Streeter (1924) as due to the retention of hydrogen sulfide by the casein in solution. The continued increase of soluble arsenic in solution on addition of larger quantities may be explained by assuming that the arsenic acid formed in the reaction will further react upon the lime sulfur solution, causing the release of more hydrogen sulfide.

QUADRUPLE MIXTURES CONTAINING LIME SULFUR, LEAD ARSENATE, LIME AND DRIED SKIM MILK.—Table 8 shows the analyses of mixtures containing various quantities of lime and dried skim milk when added to a standard preparation of lime sulfur, diluted 1–50, and lead arsenate in the proportion of 3 pounds in 100 gallons of solution. All of the mixtures were prepared according to the standard procedure and the solid materials added in the proportion of pounds in 100 gallons as indicated.

The addition of dried skim milk to the mixture containing 8 ounces of lime reduced the sulfide content of the solution. As the proportions were increased through 1, 2 and 3 pounds, the sulfide sulfur in the mixture continued to decrease, with a total loss in percentage of approximately 12 per cent. The mixtures containing equivalent quantities of lime and dried skim milk showed the best retentive power in respect to the sulfide sulfur in solution. The protective action of the lime in the prevention of the formation of soluble arsenic was retarded by the addition of dried skim milk, and the soluble arsenic found in the 8–3–$\frac{1}{2}$–3[4] mixture was similar to that found in the double mixture of lime sulfur and lead arsenate. The molecular ratio of sulfur to calcium, indicating the condition of the polysulfides in the solution, was increased as the proportion of dried skim milk was increased in the mixture.

Similar results were shown in analyses of mixtures containing various proportions of skim milk in the dry form to the mixture of lime sulfur, lead arsenate and 1 pound of lime. However, the addition of 8 ounces of skim milk powder was less effective in this mixture than 1 pound. Mixtures containing 2 and 3 pounds of dried skim milk to 1 pound of lime showed a decrease in the sulfide concentration below that found in solutions made from mixtures containing equal quantities of lime and dried skim milk. The protective influence of lime with respect to the soluble arsenic formed in the mixtures was only slightly interfered with by the addition of dried skim milk in quantities of 8 ounces, 1, 2 and 3 pounds. The ratio of sulfur to calcium in the sulfide form as calculated on the solutions decreased as the quantities of dried skim milk was increased.

Analyses of the mixtures containing lime sulfur, lead arsenate and 2 pounds of lime with varying proportions of dried skim milk are also shown in table 8. The results with this series of mixtures were similar to those of the previous experiments, with the exception that the addition of dried skim milk in proportions of 8 ounces, 1 and 2 pounds increased the retention of the sulfide concentration of the mixtures with the 8–3–2–2 mixture, showing the maximum retention of the sulfide concentration. The mixtures containing 3 pounds of dried skim milk showed a decrease in the sulfide percentage when compared to 8–3–2–2 mixture.

The mixtures prepared with 3 pounds of lime and varying quantities of dried skim milk (table 8) showed a continuous increase in the percentage of sulfide sulfur in solution as the amount of dried skim milk was increased in the proportion of 8 ounces, 1, 2 and 3 pounds with the 8–3–3–3 mixture showing the maximum retention of the sulfide concentration.

Table 9.—Percentage sulfide concentration in triple and quadruple mixtures containing lime sulfur, diluted 1–50, three pounds of lead arsenate and various proportions of lime and dried skim milk in 100 gallons of solution. Calculations are based on the total sulfur added to the solution. Lime sulfur solution, diluted 1–50, showed a sulfide concentration of 93.87 per cent.

POUNDS LIME	POUNDS DRIED SKIM MILK				
	0	½	1	2	3
0	70.25	72.09	73.73	73.90	74.46
½	88.18	87.25	82.32	80.14	76.22
1	80.01	83.04	86.89	84.86	81.75
2	77.56	80.36	82.08	86.72	83.42
3	76.04	78.67	80.28	85.57	86.40

Table 9 indicates the sulfide concentration found in the various quadruple mixtures, calculated on the basis of the total sulfur added to the solution as lime sulfur solution at a dilution of 1–50. Since the total sulfur in the mixtures containing lime sulfur and lead arsenate and the triple mixtures containing lime sulfur, lead arsenate and dried skim milk was reduced by precipitation of sulfur from the solution, the sulfide concentration as indicated in table 9 for these mixtures is less than those calculated in tables 3 and 7.

On the basis of the sulfide concentration of the solution, a mixture of the proportion of 8–3–1–3 was just as effective as a mixture containing the components in proportion of 8–3–3–1. However, the former mixture indicated a greater ratio of sulfide to sulfide calcium in the solution. If, for other considerations, it is

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[4] In the proportions used here and subsequently throughout this paper, the first figure represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked lime; and the fourth, pounds of dried skim milk; all figures are on the basis of 100 gallons of spray mixture

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desirable to add dried skim milk powder, from the analyses it appeared that the proportion of 8–3–$\frac{1}{2}$–$\frac{1}{2}$ was the best from the point of view of the retention of the sulfide concentration, the lowering of the amount of soluble arsenic in solution and prevention of formation of large quantities of residue precipitated from the quadruple mixtures. However, the proportion 8–3–3–3 produced a relatively small amount of soluble arsenic with a relatively large amount of sulfide sulfur.

COMPARISON OF VARIOUS COMBINATIONS.—To facilitate comparison, the various combinations investigated in this study have been arranged in the form of two charts (figs. 3 and 4), in which the amounts of polysulfide sulfur (as per cent of the total sulfur present) and the soluble arsenic (as grams per 100 gallons of spray mixture) are shown.

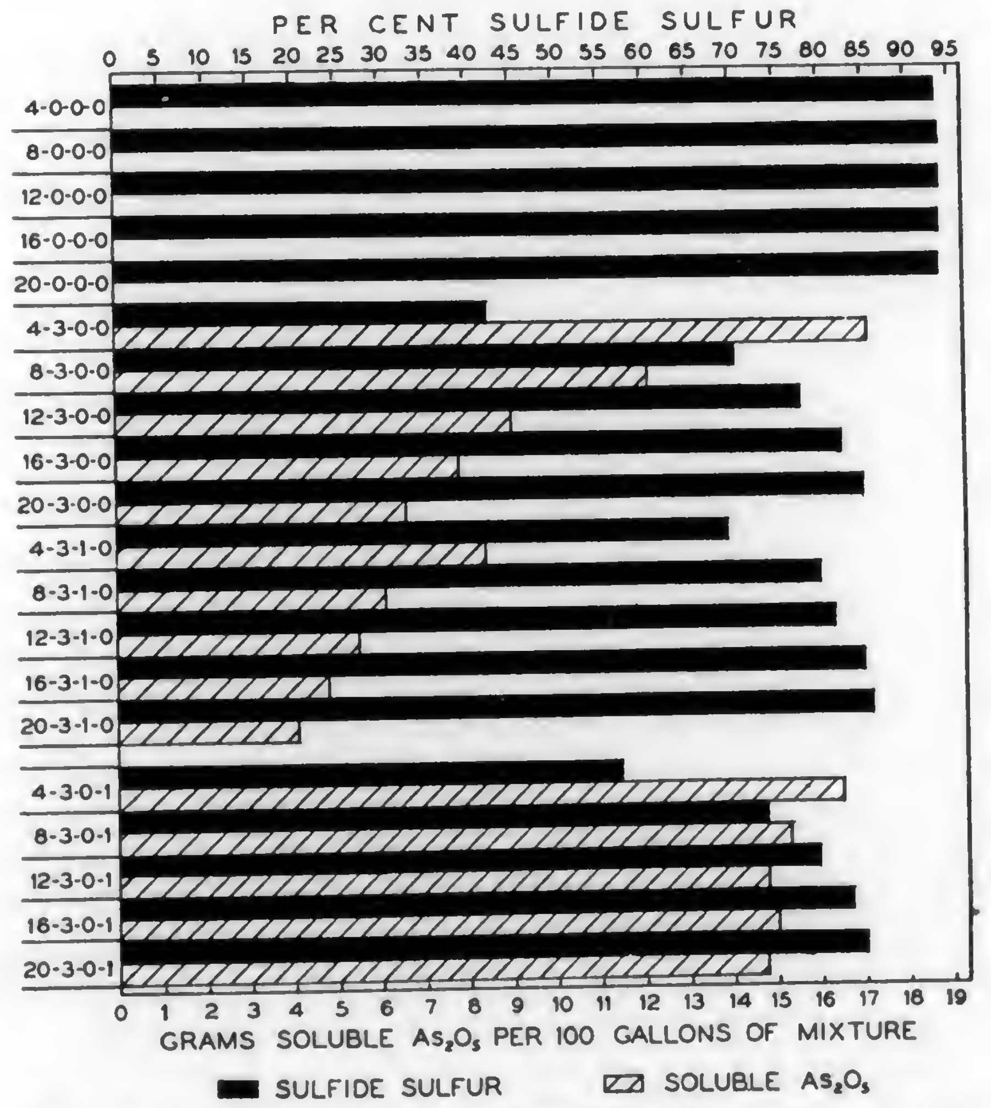

FIG. 3.—Sulfide sulfur and soluble As_2O_5 in various mixtures. The first digit in the left column represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked line; and the fourth, pounds of dried skim milk; all figures are on the basis of 100 gallons of spray mixture.

In considering the data presented in these figures, it is interesting to note that Swingle (1929) found that a concentration of As_2O_5 in a spray mixture equivalent to approximately 4.5 grams (0.0012 per cent) per 100 gallons did not injure peach foliage, but that 5.7 grams (0.0015 per cent) of As_2O_5 per 100 gallons caused slight leaf burning on peach foliage. The apple is considered more resistant to arsenical injury than the peach, so presumably the limits would be higher for this fruit.

It should be pointed out that in the quadruple mixtures, which are shown in the last four groups of fig. 4, the general effect on the percentage of polysulfide sulfur of the various proportions seemed to be rather slight, with variations only from 87.5 to 77.0 per cent, although the consistent differences within each group are to be noted. The maximum amounts found are the same within the experimental error, and appeared in mixtures with the proportions 8–3–$\frac{1}{2}$–$\frac{1}{2}$; 8–3–1–1; 8–3–2–2 and 8–3–3–3. These are the mixtures in which the lime and skim milk powder were in equivalent amounts. The soluble arsenic, on the other hand, showed

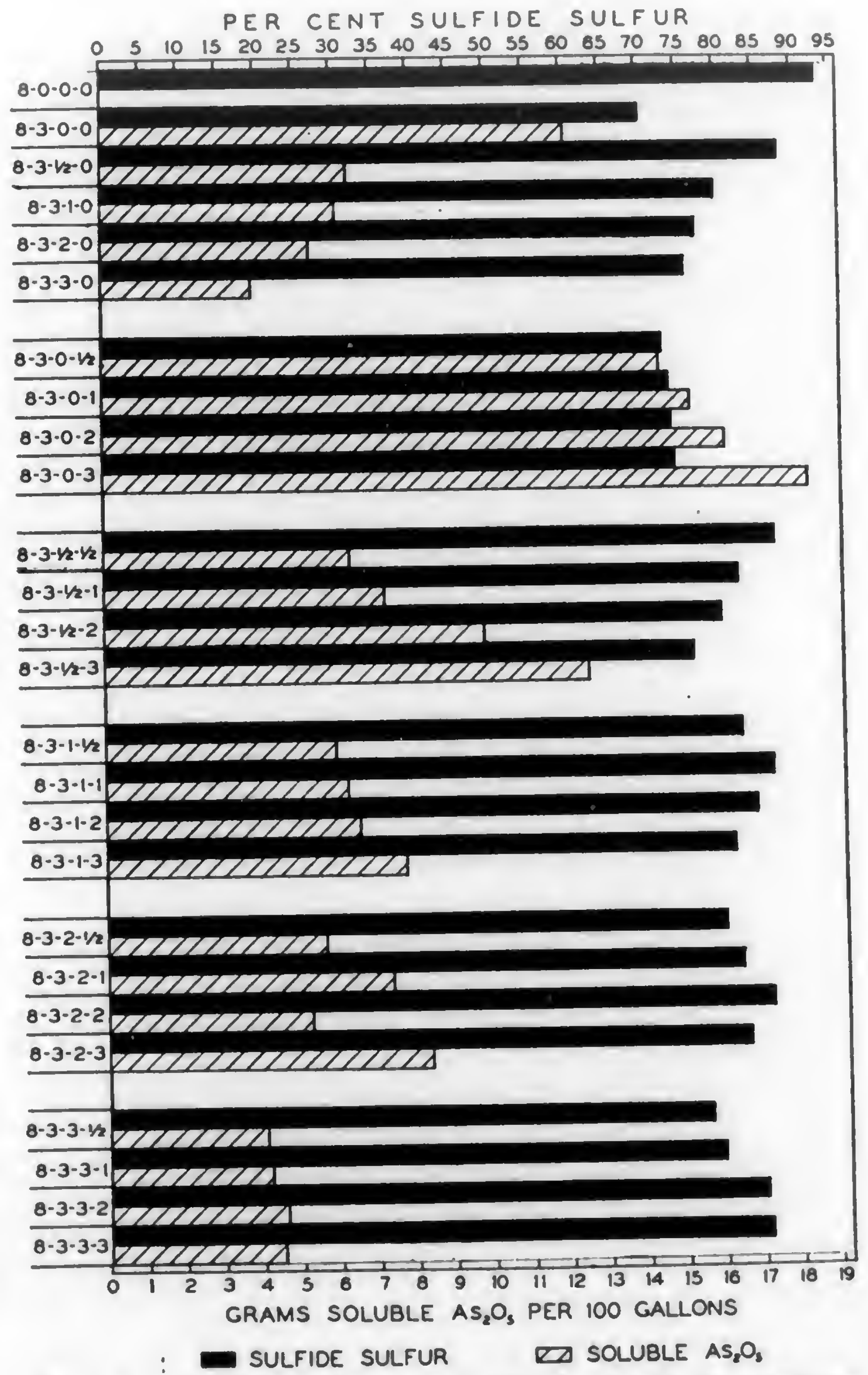

FIG. 4.—Sulfide sulfur and soluble As_2O_5 in various mixtures. The first digit in the left column represents quarts of lime sulfur concentrate; the second, pounds of lead arsenate; the third, pounds of slaked lime; and the fourth, pounds of skim milk; all figures are on the basis of 100 gallons of spray mixture.

rather wide variations, the minimum amounts (within the limits of the experimental error) being found in the mixtures with the proportions 8–3–3–$\frac{1}{2}$; 8–3–3–1; 8–3–3–2 and 8–3–3–3. The difference between the amounts of soluble arsenic in these mixtures and that found in the 8–3–$\frac{1}{2}$–$\frac{1}{2}$ combination is slight, and probably not significant from a practical standpoint when such mixtures are used on apple trees. This is of considerable practical importance when the cost of materials is to be considered.

CONCLUSIONS.—Over a one hour period, lime sulfur solution was stable toward oxidation in solutions more concentrated than 1–100. The solutions contained quantities of soluble sulfur, the amount of which depended upon the dilution of the concentrate.

A period of 45 hours was necessary for the decomposition of the sulfide sulfur in a lime sulfur solution diluted 1–50. Thiosulfates and sulfur as the sulfate were formed in solution as the sulfide disappeared.

Distilled water used as the diluent gave the most consistent results on analysis of the lime sulfur solution, but in general any of the waters tested may be used for dilution without change in the soluble sulfur in solution.

Decomposition of lime sulfur by the addition of lead arsenate was found to be greater in the dilute mixtures than in the concentrated. In solutions more concentrated than 1–50, the decomposition appeared to be a constant quantity.

The effect of a change in temperature upon the decomposition of lime sulfur by lead arsenate appeared to be only as an alteration of the rate of decomposition.

Arsenic appeared to be rendered soluble in the solution; the amount of which was relative to the quantity of hydrogen sulfide evolved from the solution.

Lime added to mixtures of lime sulfur and lead arsenate prevented the decomposition of the sulfide sulfur of the solution. The arsenic in solution was decreased as the proportion of lime was increased. Residues resulting from the addition of 1 pound of lime to the diluted mixtures of lime sulfur and lead arsenate between the range of 1–20 and 1–100 were constant. The reduction in weight of the residue approximated the weight of lead sulfide present in residues from mixtures of similar dilution containing lime sulfur and lead arsenate.

Progressive increase in the proportion of lime added to a 1–50 dilution of lime sulfur and lead arsenate showed a varied effect upon the percentage of sulfide sulfur in solution. When 8 ounces of lime were added, a protection from decomposition of the sulfide resulted which was not equaled by any quantity used until 9 pounds of lime were added. The condition of the sulfides with respect to calcium in combination in solution was decreased as lime was added to the mixture.

Dried skim milk in combination with the lime sulfur and lead arsenate aided in the retention of the sulfide sulfur content of the solution. The retardation of the decomposition was not as great as that afforded by lime. Soluble arsenic was formed in the solution in quantities greater than those found in solutions from lime sulfur and lead arsenate mixtures.

Quadruple mixtures containing lime sulfur, lead arsenate, lime and skim milk in the dry form in proportions of 8 ounces, 1, 2 and 3 pounds in 100 gallons of solution showed a protection from decomposition of the sulfides, the amount of which depended upon the proportion of lime and dried skim milk that was present in the mixture.

A compilation of the percentages of sulfide sulfur found to be present in the solutions from the quadruple mixtures indicates that mixtures containing equivalent quantities of both lime and dried skim milk will give the greatest protection to the lime sulfur from decomposition by lead arsenate. The percentage of sulfide sulfur, however, did not attain the values found in solutions of lime sulfur at the same concentration.—3-18-38.

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Storage of Vitamin D in the Tissues of Growing Calves.*

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Numerous researches have been conducted with the view of determining the influence of varying intakes of vitamin D on the antirachitic potency of milk and eggs. Very few data are available, however, relative to the influence of vitamin D intake on the storage of this vitamin in the body tissues of various types of animals.

Metz and Coppens[1] have reported that some of the parenchymatous tissues from dogs contain considerable quantities of vitamin D. DeVaney and Munsell[2] have reported on the vitamin D content of ox, lamb, swine and calf livers, as purchased on the open market. Heymann[3] investigated the storage of vitamin D in various tissues of the rabbit, as resulting from feeding definite unitage of this vitamin in the form of viosterol in oil. According to this investigator, the amount of vitamin D storage was relatively small in all cases, the greatest storage being in the liver and in the blood, respectively.

It is highly probable that the lack of data concerning the ability of the larger animals to store vitamin D in their tissues is due to the amount of time and expense involved in carrying out any such investigations. Investigations of this type necessarily require an accurate measure of the total vitamin D intake of the particular animal while being maintained under carefully controlled conditions during a reasonable period previous to the time of slaughtering. Fortunately, in the investigation herein reported, an unusul opportunity for carrying out an investigation of this type presented itself when the Department of Dairy Husbandry completed some experiments which had been designed to determine the vitamin D requirement of growing calves.[4] During the above investigations a careful record had been kept of the total vitamin D intake and of the changes in body-weight of young calves from 2 weeks to 7 months of age. When the above animals were slaughtered at the end of the investigation, the tissues for the following studies were obtained.

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[1] Metz, G. A., and Coppens, Ph. A., *Nederland. Tijdschr. v. Geneesk.*, 1934, **78**, 769.

[2] DeVaney, G. M., and Munsell, H. E., *J. Home Ec.*, 1935, **27**, 240.

[3] Heymann, Walter, *J. Biol. Chem.*, 1937, **118**, 371.

Experimental. The calves from which the tissues used in these studies were taken were of the Holstein breed and were approximately 2 weeks of age when placed on experiment. These animals were maintained at all times in individual stalls in a darkened barn. Each calf was fed a diet consisting of a mixture of ground grains and skim milk powder and, in addition, received some roughage either in the form of night harvested dehydrated alfalfa hay or beet pulp. The various ingredients of the diet were so chosen as to formulate a mixture as low as possible in vitamin D but complete in all other known dietary respects. Each ingredient was subjected to biological assay for its vitamin D content previous to being incorporated into the ration.

In addition to this vitamin D-deficient basal diet, each calf, except those retained as negative controls, received carefully measured quantities of vitamin D as a dietary supplement. Some of the calves received the vitamin supplement in the form of a cod liver oil concentrate while other calves received the supplement in the form of irradiated yeast.

The length of time during which vitamin D supplements were fed before the animals were slaughtered varied somewhat with the different animals, ranging from 164 to 210 days. At the end of these periods the various animals were slaughtered in the usual manner and the desired samples taken for assay.

In preparing the samples for assay, the various tissues were taken from the animal body (in whole) as soon as possible, freed of connective tissue, weighed, macerated by passing through a meat chopper, immediately frozen and stored in this condition until ready for use. In the preparation of blood samples, an indefinite volume of the fluid (about 1000 ml) was collected in an oxalated glass container and stored at a low temperature until reduced to a suitable state for the feeding tests.

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[4] *Research Bulletin* 364, Pennsylvania Agr. Exp. Station.

From calves selected from the first series of studies, the liver, heart, brain, kidneys and a part of the blood were retained for biological assay. At first, all of the above tissues except the blood were fed in the original state in sufficiently small daily portions to insure complete consumption. However, it was soon found to be impossible for the test animals to consume sufficient quantities of the fresh tissues to bring about a satisfactory antirachitic response. It was necessary, therefore, to desiccate these tissues in order to overcome this difficulty. Even after dehydrating the tissues, it was still impossible to make an accurate determination of the antirachitic value of brain, heart and kidneys because of the inadequate consumption of these tissues by the test animals. Therefore, from those calves selected from the second series of experiments, only the blood and liver were retained for biological assay.

In order to obtain the vitamin D from the blood and to preserve it in a suitable state for the feeding tests, a fat extraction according to the Rose-Gottlieb procedure was made and the combined ether extracts were reduced to a definite volume, which was proportional to the volume of the original blood used.

All biological assays were carried out according to the U. S. P. technic[5] (1936) of assaying for vitamin D.

The data obtained as the result of determining the antirachitic potency of the bloods and livers from the various calves are given in Table I.

The data presented in the table readily disclose the lack of efficient storage of vitamin D in certain tissues of the calf. In fact, variations in the daily vitamin D intake of as much as twenty-two-fold resulted in only a threefold variation in the antirachitic potency of the bloods and a twofold variation in the antirachitic potency of the livers of the respective calves.

While a definite relationship between the vitamin D intake of the various calves and the amount of this substance found in the blood and in the liver of the respective calves does not appear to exist, the data obtained do indicate that, in most cases, those animals which received the greatest daily intake of vitamin D also stored the greatest amount of the antirachitic substance in their tissues. However, there appeared to be some exceptions to this generalization. In fact, those animals which were selected from the first series of experiments (Calves Nos. 367, 369, 381, 383, and 384) appear to have stored a lower percentage of the vitamin D in their tissues than did animals of the second series (Calves Nos. 409, 410, 411, 416, 419, 421, and 424). A possible explanation for this apparent difference

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[5] *U. S. Pharmacopoeia*, Eleventh Decennial Revision, 1936, p. 482.

TABLE I.
Vitamin D Intakes of Calves During Experimental Periods and Storage of This Vitamin in Blood and Liver.

No. of calf	Duration of feeding test, days	Gain in wt, lb	Supplementary source of Vit. D	Total Vit. D intake, U.S.P. units	Vit. D per 100 ml blood, U.S.P. units	Vit. D per 100 g liver, U.S.P. units	Total Vit. D in blood,* U.S.P. units	Total Vit. D in liver, U.S.P. units
367	164	255	Oil	809,045	13	17	1481	386
369	174	175	,,	110,770	7	15	612	265
381	164	255	None	129,695	11	13	1350	282
383	164	254	Yeast	805,810	15	19	1704	444
384	164	148	None	119,865	9	11	805	214
409	182	106	,,	39,910	17	—	988	—
410	210	253	Yeast	249,095	21	22	2645	579
411	210	214	Oil	71,005	17	17	1678	361
416	210	200	Yeast	153,785	20	15	1988	328
419	210	166	,,	77,495	15	20	1243	449
421	210	190	Oil	130,240	20	22	1750	296
424	203	180	None	48,700	—	17	—	352

*Total volume of blood calculated from the following equation:

$$\text{Vol. of blood in mls.} = \frac{\text{Wt. of calf (in lbs.)} \times 453.6 \times 0.0771}{1.059}$$

in the ability of the 2 groups of calves to store vitamin D in their tissues may be that the 2 groups of calves were born in slightly different seasons of the year. Those calves comprising the first series were born in late November, while those of the second series were born during the first half of September. If this difference in time of birth accounts for the variations in the response of the 2 groups of calves, it is possible that such a variation was due to a combination of the prenatal effect of the antirachitic condition of the cow on its offspring and to differences in the antirachitic potency of the liquid milk which the respective calves received during the first 2 weeks of life. As a whole, those calves comprising the second series received a lower intake of vitamin D during the experimental period than did those of the first series and, in turn, were able to store a larger percentage of this substance.

The 2 sources of vitamin D used in these investigations appear to be equally effective so far as storage of the antirachitic potency in the blood and in the liver of the calf is concerned.

An interesting point to be observed from the above data is the comparatively uniform concentration of vitamin D in the blood and liver of the calf. In fact, in the young of this species it appears that the liver is not an important storage organ for vitamin D. It seems, therefore, that unless there are other tissues of the calf which serve as more efficient storage organs for vitamin D than the liver or the blood, the ability of the calf to store vitamin D is rather limited. These data also indicate that the calf should be relatively susceptible to rickets if it were maintained for any extended period of time on a diet comparatively low in vitamin D and without access to ultraviolet radiation. However, serious outbreaks of rickets among calves maintained on the usual high grade rations have not been reported and in all probability have not been observed because of the appreciable antirachitic content of the sun-cured hays, roughage, and other constituents comprising a diet of this type.

Summary. A study was made of the effect of feeding young calves definite quantities of vitamin D, in the form of cod liver oil concentrate or irradiated yeast for periods of time ranging from 164 to 210 days, on the resultant storage of this substance in the blood and in the liver of the animal. From the data obtained in this study, it is concluded that only a relatively small percent of the antirachitic intake of the calf is stored in the blood and in the liver. The concentration of the antirachitic factor per unit weight was found to be approximately equal in the 2 tissues studied. Natural vitamin D as found in cod liver oil and activated ergosterol as found in irradiated yeast seem to be stored at about the same degree of efficiency.

The Biological Standardisation of the Vitamins. By KATHARINE H. COWARD, D.Sc., Reader in Biochemistry, University of London. William Wood and Company, Mt. Royal and Guilford Avenues, Baltimore, Maryland, 1938. viii + 227 pp. 44 figs. 15 × 22.5 cm. Price, $4.50.

The author, who is Head of the Nutrition Department of the Pharmaceutical Society of Great Britain, is a recognized authority on methods of vitamin assay. It is fitting, therefore, that the first book to be published in English covering all essential details of biological determinations of vitamins, should come from the pen of one who has contributed so much to the establishment of our present international standards of vitamin unitage.

As the title suggests, the book is devoted exclusively to questions involving the quantitative accuracy of vitamin assays. She has confined her discussion to those vitamins (A, B_1, C and D) the methods for which have been standardized and which have received the approval of the International Vitamin Standards Committee of the League of Nations.

The book is divided into two parts. Part I is devoted to practical phases of vitamin work and may be used without reference to Part II. The latter has to do with the biometrical phases of vitamin work and emphasizes the value of statistical treatment of assay data.

Part I contains seven chapters dealing with such topics as "General Principles which Govern the Biological Methods for the Determination of the Vitamins," "Animals Suitable for the Determination of Vitamins by Biological Methods," "The Determination of Vitamin A, B_1, C and D" (one chapter devoted to each vitamin) and "The Interdependence of the Vitamins."

Each chapter which deals with the determination of a vitamin is preceded by an outline of the material which is to be discussed. The following outline of Chapter III, "The Determination of Vitamin A," is typical of the plan and treatment followed in discussing vitamin assay problems:

1. The International Standard of Reference and Unit of Vitamin A Activity.
    - A. The dilution of the standard of reference for dosing.
    - B. The need for a simultaneous test of the Standard whenever a determination of vitamin A is made.
    - C. The general arrangement of the test for a determination of the vitamin A potency of a substance in terms of the International Standard.
2. The Preparation of Rats for Vitamin A Determinations.
    - A. Animals suitable for the test.
    - B. Housing of the animals.
    - C. Vitamin A-free diet.
3. Criteria for the Measurement of the Response of Rats to Doses of Vitamin A.
    - A. Increase in weight.
    - B. Occurrence of xerophthalmia.
    - C. Changes in the vaginal contents.
    - D. Comparison between prophylactic and curative methods.
    - E. Comparison between (a) the "increase in weight" method, (b) the "xerophthalmia" method and (c) the "vaginal contents" method.
4. Physical Properties of Vitamin A by means of which it may be Measured.
5. References.

Part II consists of five chapters dealing with "The Standard Deviation" and "The Accuracy Obtainable in Determinations of Vitamin A, B_1, C and D." An appendix consisting of ten pages contains a report of the Second International Conference on Biological Standardisation and a table of Vitamin Content of Some Common Foods, expressed in International Units.

The author shows remarkable familiarity with the various methods and laboratory practices prevailing in the best European and American laboratories. Even the experienced worker will find Part I (practical) of great value and it is the reviewer's opinion that every vitamin assay laboratory should have the book available, since Dr. Coward has brought together material which is widely scattered in the literature.

Part II, however, contains information that is even less readily available than that referred to in Part I. Furthermore, most workers are not familiar with the use of statistical methods. Dr. Coward has the happy faculty of making statistical methods usable and understandable for the average worker.

The author has shown excellent judgment in her choice of photographs, tables and charts which illustrate the points emphasized in the text. No worker in vitamin research can afford to be without this very useful book.

R. Adams Dutcher

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THE RELATION OF PLANT MATURITY TO THE TYPE OF ORGANISMS PREDOMINANT IN PLANT DECOMPOSITION[1]

D. W. McKinstry, D. E. Haley, and J. J. Reid[2]

That the growing plant harbors a large population of microorganisms has long been recognized. More than forty years ago the concept of bacterial epiphytes was advanced and received much attention. Possible relationship of this flora to bacterial plant pathogens was advanced by certain workers but vigorously denied by specialists in the field of plant pathology. Relationship of the flora of the living plant to that of the decomposing plant proved of interest to a few investigators during the past half century.

Our knowledge of this subject today may be summarized briefly. Plants support a flora consisting largely of Gram-negative rods, the types of which are limited in number. Aerobic spore-forming bacteria, micrococci and fungi are ordinarily present in relatively small numbers in comparison with the population of Gram-negative rods. Possible relationship of the Gram-negative rods to bacterial plant pathogens is still unknown. The relative importance of Gram-negative rods and aerobic spore-formers in the initial stages of the decomposition of plant material is still a matter of dispute.

Some of the major problems confronting the growers and manufacturers of Pennsylvania cigar-leaf tobacco are bacteriological in nature. For example, the bacterial disease commonly known as "Wildfire" is serious in Pennsylvania. Unsatisfactory fermentation of the cured leaf is not uncommon. Spoilage in curing shed and warehouse is discouraging to grower and manufacturer alike.

In an effort to solve some of these problems it seemed advisable to make a study of the flora of the tobacco leaf from seed bed to manufacture and attempt to relate the activity of microorganisms to the chemical composition of the substrate--the tobacco leaf. This brief report covers that part of the program pertinent to the relationship of plant maturity to microbial activity.

The plant material used in this study was obtained from soil fertility plots of The Pennsylvania State College and the United States Department of Agriculture located in Lancaster County, Pennsylvania, and from growers and buyers of cigar-leaf tobacco in the Lancaster district. The samples represented cigar-leaf tobacco in the various stages of growth from seed-bed to harvest and samples from curing sheds and warehouses. Crops produced in 1937 and 1938 were represented. In addition, supplementary studies were conducted on red clover grown in Centre County, Pennsylvania.

Methods used in chemical analysis of the samples conformed with those recommended for plant material by the Association of Official Agricultural Chemists.

Plate counts of bacteria were made on standard nutrient agar adjusted to pH 6.8 and replicates were incubated at 20, 25, and 37 degrees C. Plate counts of fungi were made on glucose-peptone agar adjusted to pH 4.5 and incubated at room temperature. Crystal violet was used at times in dilutions of 1:200,000 in attempts to inhibit growth of Gram-positive organisms on nutrient agar. Cultural characteristics of isolations were determined in the usual laboratory manner. Bacteriological data presented are based on more than 7,000 isolations from cigar-leaf tobacco and a number from other sources.

The examination of tobacco leaves from seed-bed and field has revealed much of interest. As a result of this study it may be assumed that the normal growing leaf harbors upon its surface a flora of Gram-negative rods ranging from ten million to one hundred million per gram of tissue, dry weight. If classified according to the system in common use in this country these forms would be listed as members of *Flavobacterium*, *Pseudomonas* and *Aerobacter*. The types commonly found in abundance certainly number less than half a dozen. Physiologically this number might well be reduced to three, consisting of a rather inert type, an acid and gas producer upon carbohydrate media, and a very proteolytic form which also ferments a few carbohydrates with the production of gas. The last type may or may not produce a green soluble pigment; the two former may or may not produce a yellow pigment. The physiological resemblance of these forms to some of the plant pathogens is striking.

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[1] Authorized for publication on November 28, 1938, as paper No. 866 in the Journal Series of the Pennsylvania Experiment Station, State College, Pa.

[2] Graduate Scholar, Phytochemist and Soil Bacteriologist, respectively.

Aerobic spore-formers were found present in numbers ranging from a few thousand to several hundred thousand per gram dry weight. The predominant members of this group were identified as *Bacillus megatherium*, *Bacillus cereus*, and related organisms.

Micrococci were found present in lower numbers than the spore-formers. Both pigmented and non-pigmented forms were usually noted.

The technique employed in determining molds revealed numbers per gram of from 50,000 to 200,000. The genera *Penicillium*, *Aspergillus*, and *Fusarium* were most often seen.

By use of a chamber in which both temperature and relative humidity were under control, decomposition of the green leaf was studied. Plate counts were made and colonies were picked during the progress of the decomposition. Two types of bacteria were always found in great numbers, the gas producer and the proteolytic fluorescent type found on the normal leaf. Gram-positive organisms apparently played little if any part in the process.

A study of the flora of the normal red clover leaf showed great similarity in numbers and forms to the flora of the green tobacco leaf and decomposition of the green leaf was similar to that of the tobacco leaf.

The flora of the normal green tobacco leaf having been established, attention was turned to an attempt to ferment cured immature leaves. It at once became apparent that samples of such material differed markedly in the nature of the fermentation possible. Immature leaves harvested early in the season and cured during the warm weather of late summer under favorable curing conditions were found to undergo in most cases the Gram-positive fermentation of normal cigar-leaf tobacco. On the other hand, immature leaves harvested late in the season and cured during the cool weather of fall were found to undergo a Gram-negative decomposition in which the predominant organisms proved to be those of the decomposing green leaf.

Attempted fermentation of rapidly desiccated mature leaves, omitting the normal cure of the leaf, resulted at times in Gram-negative decomposition, at times in normal Gram-positive fermentation and at times in the development of a mixed flora and an unsatisfactory end product.

The change of the substrate from a type favoring the activities of Gram-negative organisms to one favoring the Gram-positive organisms would appear to depend to a certain extent upon two factors, plant maturity and a cure involving the slow death of the plant cells by starvation.

In an effort to relate the bacterial activity to chemical composition of the substrate a number of analyses were made. The chemical data obtained may be summarized in a few sentences. Plant tissue that favored the development of Gram-negative rods in fermentation was found to possess a significant content of fermentable carbohydrates, particularly starch. Such tobacco was also found to have a relatively low content of fixed organic acids. The nitrogen fraction was of particular interest inasmuch as the protein content was relatively high and the amide content low.

On the other hand, tobacco that permitted the development of a satisfactory flora of aerobic spore-formers and micrococci was found to have an insignificant content of fermentable carbohydrate material, a high percentage of fixed organic acids and a nitrogen distribution essentially different from that of the improperly fermenting tobacco. In this case the protein content was lower than in the case of green tobacco and the amide content was higher.

Summary

A characteristic flora was found on the leaves of growing cigar-leaf tobacco. A similar flora was found on red clover.

The predominant forms found upon the growing plant were Gram-negative rods. Aerobic spore-formers, micrococci and fungi could always be demonstrated though present in relatively small numbers.

Decomposition of green tobacco was found to be associated with the activities of the Gram-negative rods.

Immature cigar-leaf harvested late in the season was found to be generally characterized by a Gram negative decomposition in any attempt at fermentation. Immature cigar-leaf harvested early in the season and cured during the late summer was found to ordinarily develop a Gram-positive flora and ferment satisfactorily.

Mature leaf improperly cured was found

to develop a satisfactory Gram-positive flora in some cases, and a Gram-negative flora in others.

Both maturity and cure seem important in the change of the substrate from a type favoring the development of Gram-negative rods to a type permitting the development of Gram-positive organisms.

Soluble carbohydrates and high protein content were found to be associated with Gram-negative development.

Absence of soluble carbohydrates, reduced protein content, and a high content of fixed acids and amides were chemical characteristics found associated with the development of a Gram-positive flora.

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Plot No.	Relative Quality	Y. A
1	poor	
2	fair	
3	good	1
4	good	1
5	good	1
6	good	1
7	good	1
8	fair	1

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THE EFFECT OF FERTILIZER TREATMENT UPON THE TYPE OF FLORA FOUND UPON DECOMPOSING PLANT TISSUES[1]

R. G. Harris, D. E. Haley, and J. J. Reid[2]

The presence of large numbers of viable microörganisms upon a fermentable substrate that is undergoing decomposition usually suggests the possibility that the microorganisms may be responsible for the chemical changes taking place. Such is the case in the aging of flue-cured tobacco, the changes in which, though slow, are by their very nature suggestive of bacterial metabolism. With this in mind the flora of flue-cured tobacco has been studied.

A preliminary survey showed that a significant number of microörganisms survived the flue-curing process and multiplied upon the tobacco during the subsequent storage period on the farm. The types found included Gram-negative rods, micrococci, sporeformers, molds and yeast-like organisms, but of these the Gram-negative rods were ordinarily present in greatest numbers.

The types of Gram-negative rods present, however, were found to vary with samples received from various sources and in an effort to account for this arrangements were made to receive tobacco produced under carefully controlled conditions of soil fertility. A study of these samples showed that with potash the only variable in a series of treatments the effect upon the microflora of the leaf was surprisingly great. The data obtained in this study are presented in this paper.

Materials and Technique

The chemical and bacteriological determinations reported were made upon tobacco procured from the Chatham Substation of the Virginia Agricultural Experiment Station through the courtesy of E. M. Matthews. The samples represented flue-cured tobacco produced in 1937 on plots treated with thirty pounds of nitrogen, eighty pounds of P_2O_5 and with varying amounts of K_2O as shown in Table 1. The samples represented the fifth priming and consequently were not representative of the best quality produced on the plots. On the other hand, the effect of the various treatments upon yield and quality was pronounced in this priming as in the others. Yields and crop values, through the courtesy of Mr. Matthews, are presented in Table 2. Samples for the determinations were taken at the time the crop was marketed.

Table 1*

FERTILIZER TREATMENTS OF THE TOBACCO PLOTS AT THE CHATHAM SUBSTATION

Plot No.	Drilled in Furrow at Rate of 1000 Pounds per Acre	Side Dressing of K_2O after Planting	Total K_2O in Treatment
		lb.	lb.
1	3-8-0	0	0
2	3-8-3	0	30
3	3-8-6	0	60
4	"	30	90
5	"	60	120
6	"	100	160
7	"	140	200
8	"	240	300

*Data presented in this table were obtained from E. M. Matthews, superintendent of the Chatham Substation of the Virginia Agricultural Experiment Station.

Other samples from which supplementary information has been obtained were received from the North Carolina Experiment Station through the courtesy of E. G. Moss and from a number of growers in the flue-cured district. The samples received from these sources were largely from the 1937 crop, although a few from the 1938 crop have been included. The co-operation of the American Potash Institute made possible the receipt of this material.

Methods used in the chemical and bacteriological determinations were those best adapted to plant material such as tobacco.

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Table 2*

EFFECT OF FERTILIZER TREATMENTS UPON QUALITY AND YIELD OF TOBACCO PRODUCED ON THE CHATHAM PLOTS

Plot No.	Relative Quality	Yield per Acre lb.	Value per Acre	Price per Pound
1	poor	821	$176.03	21¢
2	fair	977	236.72	24
3	good	1229	333.74	27
4	good	1155	350.46	30
5	good	1019	275.58	27
6	good	1248	324.88	26
7	good	1194	282.86	24
8	fair	1038	205.05	20

*Data presented in this table were obtained from E. M. Matthews, superintendent of the Chatham Substation of the Virginia Agricultural Experiment Station.

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, potassium by the platinic-chloride method and moisture was determined by drying without heat over concentrated sulfuric acid to constant weight. Reducing sugars were determined by the method of Quisumbing and Thomas upon an aqueous extract of the sample.

Plate counts of bacteria were made on standard beef-extract peptone agar adjusted to pH 6.8. Preliminary investigation revealed no significant benefit from addition of glucose, asparagine or tobacco extract to this substrate. Counts of fungi were made on glucose-peptone agar adjusted to pH 4.5. All colonies of bacteria and yeast-like forms appearing on plates with counts between ten and 100 were "picked" and the isolations subjected to laboratory study.

Discussion of Results

Data obtained by chemical analysis of the tobacco samples from the Chatham potash plots are presented in Table 3. The influence of potassium upon the reducing sugar content is worthy of particular note although not unexpected. The depressive effect of potassium upon nitrogen uptake is likewise clearly demonstrated in this series of treatments in which potash is the only variable. This, too, is in accord with published results based on similar experiments.

Comparing the data presented in Table 3 with that of Table 2 it would appear that what the buyer pays for as quality in a flue-cured leaf includes a reducing sugar content as high as is compatible with a nitrogen content of the order of 2.25 to 2.50 per cent. Plots 3, 4, and 5 produced such tobacco. The low reducing sugar content and high nitrogen of the tobacco produced on Plots 1 and 2 would seem to be correlated with what the buyer would term poor to fair quality in a flue-cured tobacco. On the other hand, the tobacco produced on Plots 7 and 8 likewise failed to bring high prices and this lack of quality would seem to be associated with an abnormally low nitrogen content.

Table 3

EFFECT OF FERTILIZER TREATMENTS UPON THE CHEMICAL COMPOSITION OF THE TOBACCO PRODUCED ON THE CHATHAM PLOTS

Plot No.	Reducing Sugars as Glucose %	N %	K %	Soluble Carbohydrate/N Ratio	N/K Ratio	Ash %
1	5.97	3.12	1.63	1.91	1.91	14.06
2	7.81	2.72	1.84	2.91	1.48	12.12
3	10.10	2.47	2.43	4.08	1.02	12.30
4	11.93	2.53	2.38	4.71	1.06	11.21
5	.....	2.42	2.55	....	0.95	12.35
6	12.01	2.14	2.52	5.61	0.83	11.53
7	12.76	2.19	3.07	5.82	0.71	11.86
8	10.26	2.33	3.29	4.40	0.71	13.40

An examination of Tables 2 and 3 reveals that the eight plots fall into three distinct groups. The groups are identical whether the division be made on the basis of selling price or upon chemical composition. The first group, consisting of Plots 1 and 2, produced tobacco that sold for approximately twenty-three cents per pound. The N/K ratio of the crops produced on these plots fell between 1.48 and 1.91. The second group, comprising Plots 3, 4, and 5 produced tobacco selling on the average at twenty-eight cents per pound. The N/K ratio of the tobacco from these plots ranged from 0.95 to 1.06. The third group, consisting of Plots 6, 7, and 8, produced tobacco valued at about twenty-four cents per pound, and with an N/K ratio between 0.71 and 0.83.

Plate counts of bacteria and yeast-like organisms are presented in Table 4. The data presented in this table make it evident that bacteriologically the same grouping may be made as that made upon the basis of quality and confirmed by chemical analysis. From the standpoint of bacterial numbers such a grouping is possible although it must be admitted that the variations in count are not of great significance. The truly significant differences, however, that permit such a grouping are found in the relative distribution of types of microorganisms. The first rough breakdown of types is presented in Table 5. Upon the basis of colonial morphology of the Gram-negative rods the eight plots are found to fall into the three groups previously discussed. Gram-negative rods growing on tobacco from Plots 1 and 2 were found in almost every instance to be "rough" in nature. Gram-negative rods from tobacco produced on Plots 3, 4, and 5 were approximately fifty per cent "rough" in type and fifty per cent "smooth." All Gram-negative rods found on tobacco from Plots 6, 7, and 8 were "smooth" in colonial morphology.

Table 4

EFFECT OF FERTILIZER TREATMENT UPON THE MICROFLORA OF THE TOBACCO PRODUCED ON THE CHATHAM PLOTS AS DESCRIBED

Plot No.	Plate Count of Bacteria and Yeasts per Gram	Percentage Distribution of Principle Types of Micro-organisms			
		Gram-negative rods		Cocci	Yeast-like organisms
		Smooth	Rough		
1	103,000	0	93	7	0
2	660,000	3	81	16	0
3	2,730,000	20	76	4	0
4	1,904,000	54	28	0	18
5	4,400,000	27	19	0	54
6	675,000	86	0	7	7
7	365,000	91	0	9	0
8	830,000	100	0	0	0

Micrococci were seldom encountered on tobacco from Plots 3, 4, and 5 although characteristically present on tobacco from other plots. With the exception of tobacco from Plot 6, yeast-like forms were limited to the crops produced on Plots 4 and 5.

Of particular interest, however, and confirming the classification of the eight plots into three groups, is the study of the Gram-negative isolations recorded in Tables 6 and 7. All Gram-negative rods isolated from the tobacco grown on these plots produced a yellow pigment at the time of isolation, though pigment production has not proved constant under laboratory cultivation. At the time of "picking" these cultures were classified as "rough" and "smooth" as shown in Table 4. The cultures were subsequently transferred to gelatin, litmus milk, and glucose, sucrose, and lactose peptone broths for further classification. In this manner the "smooth" forms were subdivided into two groups or types and the "rough" forms into three as indicated in Table 6. Distribution of the Gram-negative rods on this basis is shown in Table 7. This distribution not only places additional emphasis upon the effects of the plot treatments, but again indicates a division of the eight plots into the three groups previously described. For example, Rough Type III has been found only on tobacco produced on

Table 5

EFFECT OF LABORATORY CULTIVATION UPON THE CULTURES OF GRAM-NEGATIVE RODS ISOLATED FROM TOBACCO PRODUCED ON THE CHATHAM PLOTS

Plot No.	Relative Numbers of Smooth and Rough Cultures upon Isolation		Relative Numbers of Smooth and Rough Cultures Following Laboratory Cultivation	
P	Smooth %	Rough %	Smooth %	Rough %
1	0	100	95.6	4.4
2	3.5	96.5	63	37
3	21	79	70	30
4	65	35	69	31
5	59	41	59	41
6	100	0	100	0
7	100	0	100	0
8	100	0	100	0

Table 6

CULTURAL CHARACTERISTICS OF GRAM-NEGATIVE RODS ISOLATED FROM TOBACCO PRODUCED ON CHATHAM FERTILITY PLOTS

Type	Gelatin	Litmus Milk	Glucose, Sucrose and Lactose
Smooth Type I	Pronounced liquefaction 4-7 days	Usually only reduction, rarely slight digestion	Usually alkaline, some give slight acidity
Smooth Type II	As above	Slight if any change	Acid and gas
Rough Type I	As above	Acid curd	Acid and gas
Rough Type II	Liquefaction very slow	Reduction	Usually alkaline
Rough Type III	As above	Yellow pellicle, no other change	Alkaline

Table 7

DISTRIBUTION OF THE FIVE TYPES OF GRAM-NEGATIVE RODS ISOLATED FROM TOBACCO PRODUCED ON THE CHATHAM PLOTS

Plot No.	Smooth I %	Smooth II %	Rough I %	Rough II %	Rough III %
1	..	...	55	45	..
2	3	1	18	78	..
3	21	...	1	49	29
4	53	13	..	5	29
5	41	18	..	..	41
6	30	70	..	..	..
7	..	100	..	..	..
8	15	85	..	..	..

Plots 3, 4, and 5. This is apparently the only "rough" form isolated which retains its roughness on laboratory cultivation. Smooth Type I is also characteristic of the higher quality tobacco while Smooth Type II seems more characteristic of an extremely low nitrogen tobacco. Rough Types I and II were found to be associated with rather high nitrogen content.

Continued laboratory cultivation over a period of several months has resulted in the conversion of the majority of the cultures of the Rough Types I and II to "smooth" forms and it would appear that Rough Type II is the "rough" phase of Smooth Type I and Rough Type I bears a similar relationship to Smooth Type II. This conversion is shown in Table 5.

Although laboratory cultivation has resulted in the loss of "roughness" on the part of Rough Types I and II, merging these cultures with Smooth Types II and I, respectively, it is felt to be significant that treatment of the soil with corresponding differences in plant composition resulted in such dissociation on the part of these organisms. In fact, it has proved possible to determine by plating the approximate N/K ratio of Old Belt tobacco according to the relative numbers of "smooth" and "rough" forms present. Such a determination is not possible, however, in the case of tobacco from other flue-cured districts.

The investigation of tobacco from other flue-cured districts has shown that the crop from each of the three large producing sections, Georgia, the Carolina coastal region, and the Piedmont, carries a flora characteristic of the district. The flora of the samples produced on the Chatham potash plots proved quite characteristic of the area, the predominant organism almost invariably proving to be a yellow Gram-negative rod. In lesser numbers are found on Piedmont tobacco white Gram-negative rods, micrococci, yeast-like organisms and aerobic spore-formers. Seldom have yeast-like forms been encountered in as great number as in the samples from Plots 4 and 5. These forms might well be classified as mycoderms capable of utilizing carbohydrates, many organic acids and ethyl alcohol as sources of energy. Aerobic spore-formers, as a rule, have been found to constitute a very small percentage of the flora at the end of the storage period on the farm though at other stages in the aging the numbers are more significant. These forms are largely of the *Bacillus megatherium*, *Bacillus cereus*, and related organisms. Fungi of the *Alternaria* type are always found and are usually accompanied by some of the more common molds.

Summary

Although the data at hand are based largely on a crop produced in one season, it is felt that the information presents certain significant trends.

With potash as the only variable in the fertilizer treatment the eight plots studied produced flue-cured tobacco varying in chemical composition according to the treatment.

According to the quality of the tobacco produced as judged by price received the eight plots could be placed conveniently in three groups.

Separation upon chemical analyses and bacteriological examination gave the same grouping as that based upon quality.

High quality tobacco was produced where an adequate but not excessive amount of potash was incorporated in the fertilizer treatment. The N/K ratio of such tobacco was found to be approximately 1.

"Rough" Gram-negative rods were found to be characteristic of Piedmont flue-cured tobacco that is high in nitrogen and low in soluble carbohydrates.

"Smooth" Gram-negative rods characterize the high potash, low nitrogen tobacco of the Piedmont.

Well-balanced fertilization from the standpoint of crop quality produced a tobacco upon which both "smooth" and "rough" Gram-negative rods appeared and in addition organisms not found on tobacco produced with unbalanced treatments.

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SALT CONTENT OF CURED HAM[1,2]

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The preservative action of salt has always been of primary importance in relation to the curing of ham. Recent work by Jones (1937a, b) indicates that a further function of salt lies in its selective action in addition to its bactericidal effect. Thus nitrate-reducing organisms function in high concentrations of sodium chloride which are detrimental to microörganisms having a deleterious effect on the meat.

Preservation alone does not fulfill all of the requirements of a successful cure. The further requirement for ham is for a palatable product that is not oversalted.

The present investigation was instigated because there was little definite information available to those who wish to produce a home-cured product of the best quality as judged from the viewpoint of preservation and palatability. A study was made of different curing methods from the standpoints of practicability of the method and of quality of the resulting product. This report is limited to a consideration of the salt content of hams cured by three procedures, and to a comparison of these hams with standard commercial hams obtained on the local market.

EXPERIMENTAL PROCEDURE

Forty hams were used. Of these, eight were purchased at a local butcher shop and represented first-quality products produced by nationally known meat packers. The salt content of these hams was determined for purposes of information and for comparison with our own experimentally cured hams.

Twelve hams were dry-cured under definite experimental conditions. The curing agent was composed of eight pounds of salt, three pounds of sugar, and three ounces of saltpeter (KNO_3). It was applied at the rate of one ounce per pound of meat. Usually the application of the curing agent involved rubbing the mixture into the hams in two or three portions at three-day intervals, applying as much each time to the meat as it would take up, until the required amount was used.

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[1] Presented before the division of agricultural and food chemistry at the Rochester meeting of the American Chemical Society, September 6 to 10, 1937.

[2] Authorized for publication on January 7, 1938, as paper No. 820 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Eleven hams were cured experimentally in a 75-degree (salometer) sweet pickle which was made up of the same mixture as was used for dry curing but which was dissolved in five gallons of water. The use of four gallons of water produced an 85-degree pickle which was used for the curing of nine hams.

These hams were sampled and analyzed by the methods given by Miller and Ziegler (1936) and Ziegler and Miller (1938). Five subsamples were taken from each ham (Fig. 1). Only the salt contents of the subsamples from the lean portion of the ham (No. 2, 3, 4, 5) are given in this paper; those for subsample No. 1 are omitted because this subsample usually consisted largely of fat and did not absorb salt to the same degree as did the lean tissue.

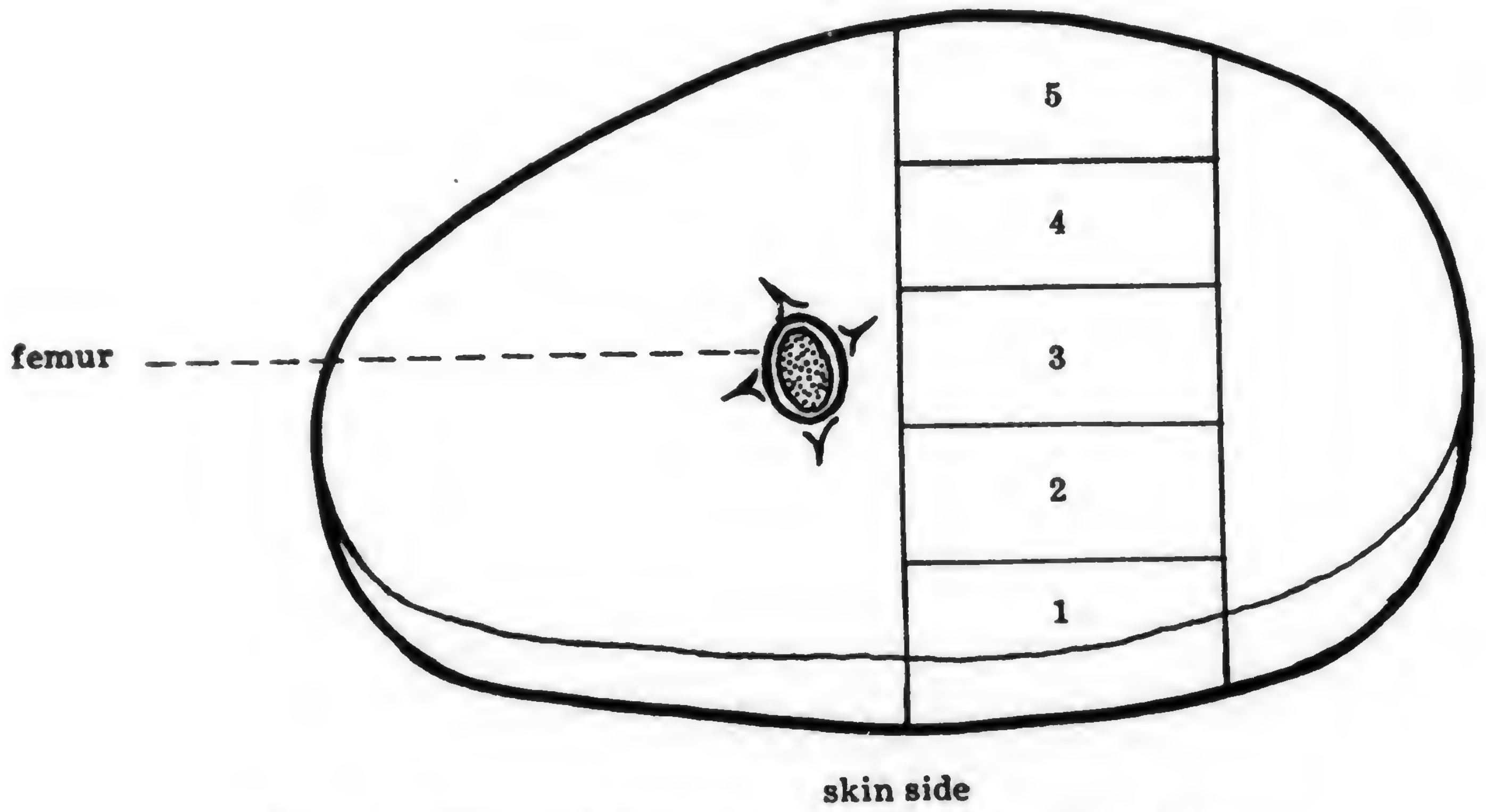

FIG. 1. Diagram showing method of sampling ham.

The method of sampling gives information as to the condition of vital portions of the ham as considered from the viewpoint of effectiveness of the cure with respect to salt penetration. The No. 3 subsample is from the center of the ham, a region next to the bone where spoilage is very likely to occur in the case of incomplete curing. The salt content of this subsample, when considered in relation to the salt content of the adjacent subsamples, gives a good idea of the extent to which salt has penetrated the ham.

SALT CONTENT OF COMMERCIAL BRANDS OF HAM

In order to ascertain what constitutes a good-quality, first-grade, commercial brand of ham as considered from its content of salt, a number of nationally known brands of ham were studied. No information is available concerning the manner in which these hams were cured but they are judged to be representative of first-quality products. These hams were sampled and the samples analyzed by the methods given above.

The data for eight such representative hams (Table 1) show that in all cases no less than 13.5 per cent of salt was present in the dry matter of the outer lean portions of these hams (Subsamples 4 and 5), and in some cases over 18 per cent was present.

Subsample No. 2, from next to the fat or skin side of the ham, and No. 3, from the center, did not contain as much salt as Subsamples 4 and 5 in spite of a possibility that the centers of these hams were pumped with a strong pickle as a part of the cure, a common commercial practice.

TABLE 1

Salt Content of Commercial Hams

Ham No.	Brand	Mean NaCl content[1] of center slice	NaCl content[1]			
			Subsample No. 2	Subsample No. 3	Subsample No. 4	Subsample No. 5
		pct.	*pct.*	*pct.*	*pct.*	*pct.*
1	A	11.63	11.44	7.45	13.68	13.92
2	A	12.91	13.67	9.99	14.43	13.54
3	A	12.51	12.16	7.91	14.50	15.46
4	B	15.75	16.53	12.87	18.06	15.52
5	B	16.35	15.88	12.63	18.36	18.54
6	B	17.42	17.73	15.56	18.49	17.91
7	C	13.97	11.09	11.22	15.41	18.14
8	C	16.18	18.14	12.21	17.65	16.73
Mean	....	14.59 ± .51	14.58	11.23	16.32	16.22

[1] NaCl in dry matter.

The mean salt content of the entire center slice, as computed from the four subsamples, ranged from 11.63 per cent to 17.42 per cent, the mean for the eight hams being 14.59 per cent.

It would appear, therefore, as judged from these hams, that 11 per cent of salt is sufficient for purposes of preservation in the center of the ham next to the bone, where spoilage is most likely to occur. It is probable that 11 per cent of salt is also sufficient to provide a considerable factor of safety in so far as the keeping quality of ham is concerned. Such a factor of safety is perhaps desirable for a commercial product which may be subjected to more or less variable conditions and hazards during storage and distribution before the meat finally reaches the consumer.

It is to be noted that, while the salt contents of all of the eight hams are of the same general order of magnitude, those of Brand A

TABLE 2

Salt Content of Dry-Cured Hams

Ham No.	Curing period	Aging period	Curing temperature	Fresh weight		Thickness	Mean NaCl content[1] of center slice	NaCl content[1]			
								Sub-sample No. 2	Sub-sample No. 3	Sub-sample No. 4	Sub-sample No. 5
	days	*days*		*lb.*	*oz.*	*in.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
1	28	30	1.1 to 2.8°C. (34 to 37°F.)	15	12	5½	9.63	8.50	8.84	9.29	11.87
2	35	30	1.1 to 2.8°C. (34 to 37°F.)	16	10	5½	9.19	8.50	8.97	8.46	10.82
3	42	30	1.1 to 2.8°C. (34 to 37°F.)	16	6	5¼	11.27	9.97	10.73	11.50	12.89
4	26	30	1.1 to 2.8°C. (34 to 37°F.)	14	1	5¼	12.48	13.13	10.30	12.50	14.00
5	40	30	1.1 to 2.8°C. (34 to 37°F.)	15	13	5½	10.87	11.25	9.69	10.71	11.83
6	28	30	Uncontrolled	14	7	4½	7.95	6.08	7.89	8.17	9.67
7	35	30	Uncontrolled	15	11	5	8.51	7.75	7.80	8.44	10.06
8	42	30	Uncontrolled	16	3	5	8.04	7.09	7.90	8.29	8.86
9	30	30	1.1 to 2.8°C. (34 to 37°F.)	14	12	4 2/7	11.24	10.02	8.70	11.23	15.00
10	35	30	1.1 to 2.8°C. (34 to 37°F.)	14	6	4 3/7	10.12	7.61	7.20	10.57	15.10
11	31	30	Uncontrolled	12	6	4 3/7	13.52	13.31	10.86	15.87	14.02
12	33	30	Uncontrolled	13	8	4 5/7	13.25	12.52	11.06	14.31	15.09
Mean	....	....	....	....	....	.....	10.51 ± .38	9.64	9.16	10.80	12.44

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[1] NaCl in dry matter.

contain somewhat less salt than those of the other two brands, indicating a somewhat milder cure for this brand.

SALT CONTENT OF DRY-CURED HAMS

Preliminary work by the authors has shown that a satisfactory dry-cure was obtained with hams when a curing formula consisting of eight pounds of salt, three pounds of sugar, and three ounces of saltpeter (KNO_3) was applied at the rate of one ounce per pound of meat. This formula or some modification of it is considered as a standard curing mixture and is recommended rather extensively for agricultural use.

Some idea of the salt content of the final product cured by this formula can be obtained by computation. Applying one ounce per pound of the 8-3-3 curing agent to a 12-pound ham containing 22 ounces of bone and 45 per cent of dry matter would result in about 11 per cent of salt in the dry matter of the ham minus the bone. However, there is some loss of salt from the ham through the brine that drains from it during dry-curing, hence 11 per cent of salt represents the maximum amount which could be present.

Data for 12 hams dry-cured by the 8-3-3 formula show that the average amount of salt in the dry matter of the center slices from which the subsamples were taken is 10.51 ± .38 per cent (Table 2). This indicates that the 8-3-3 formula gives a somewhat milder cure with respect to salt than that of the commercial hams. The average salt content of the subsample taken from the tissue next to the bone (No. 3) is 9.16 per cent, which is lower by approximately two per cent than the salt content of this subsample in the commercial hams. For Subsamples 4 and 5 differences are even greater, the dry-cured hams containing less salt by 5.52 per cent in subsample No. 4 and 3.78 per cent in No. 5. It is apparent, therefore, that the cure used for these hams gives a product which contains somewhat less salt than is found in the commercial hams used for comparison.

SALT CONTENT OF BRINE-CURED HAMS

Data for 11 hams cured in a 75-degree sweet pickle, made up as previously stated, show that the amount of salt, 10.15 ± .48 per cent, representing the average for the center slices for all the hams is not significantly different from that of the dry-cured hams (Table 3). As compared with the dry-cured hams, however, there is slightly less salt on the average in the No. 3 center subsample and somewhat more in the outside No. 4 and No. 5 subsamples. This indicates that the 75-degree pickle does not penetrate and strike into the center of the hams as rapidly as the salt does in the dry-curing. Nevertheless, the

TABLE 3

Salt Content of Hams Cured in 75-Degree Sweet Pickle

Ham No.	Curing period	Aging period	Curing temperature	Fresh weight		Thickness	Mean NaCl content[1] of center slice	NaCl content[1]			
								Sub-sample No. 2	Sub-sample No. 3	Sub-sample No. 4	Sub-sample No. 5
	days	*days*		*lb.*	*oz.*	*in.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
13	40	....	1.1 to 2.8°C. (34 to 37°F.)	15	6	$4\frac{5}{7}$	7.95	4.68	4.73	6.03	16.36
14	30	30	1.1 to 2.8°C. (34 to 37°F.)	14	2	$4\frac{3}{7}$	7.45	5.51	5.16	8.93	10.20
15	35	30	Uncontrolled	11	5	$4\frac{3}{7}$	6.35	6.70	4.43	5.98	8.30
16	45	30	Uncontrolled	15	5	$4\frac{6}{7}$	11.97	10.54	8.02	14.44	14.86
17	45	30	Uncontrolled	14	10	$4\frac{4}{7}$	10.49	9.49	8.27	11.28	12.92
18	34	30	1.1 to 2.8°C. (34 to 37°F.)	12	5	$4\frac{5}{7}$	13.04	12.19	9.74	15.50	14.73
19	50	30	1.1 to 2.8°C. (34 to 37°F.)	13	3	$4\frac{5}{7}$	12.89	8.86	10.57	13.84	18.30
20	30	30	1.1 to 2.8°C. (34 to 37°F.)	10	0	$4\frac{2}{7}$	12.87	13.78	9.85	12.72	15.13
21	30	30	1.1 to 2.8°C. (34 to 37°F.)	11	5	$4\frac{3}{7}$	10.17	10.13	9.17	11.08	11.31
22	35	30	1.1 to 2.8°C. (34 to 37°F.)	10	13	$4\frac{3}{7}$	8.43	6.08	7.65	10.25	9.72
23	34	30	1.1 to 2.8°C. (34 to 37°F.)	12	8	$4\frac{4}{7}$	10.04	8.69	6.99	12.80	11.76
Mean	....	....	....	....	....	.....	10.15 ± .48	8.79	7.69	11.17	13.05

[1] NaCl in dry matter.

saltiness of the final product is about the same as that of the dry-cured hams and is less than that of the commercial hams.

The data for the 75-degree sweet pickle hams, however, are more variable than those previously considered. This is true for samples within the individual hams as well as for samples from different hams cured by this method. While the agreement leaves much to be desired from an experimental viewpoint, the data obtained may indicate that the 75-degree sweet-pickle cure is somewhat slower in action than the dry-cure. The additional possibility remains that some of this variation may also be due to the hams themselves, uniformity of which is difficult to attain, owing to such factors as variable composition of the tissue and individual differences existing in the hogs from which the hams were taken.

The data for hams cured in an 85-degree sweet pickle indicate that the mean salt content of the center slice for all of the hams is not significantly different than that for the dry-cured and 75-degree pickle hams (Table 4). A better penetration and equalization of salt throughout the various subsamples is indicated by the mean salt contents which range from 9.61 per cent for Subsample 2 to 10.89 for Subsample 4. The salt content of the outside section (Subsample 5) of the hams is lower on the average by 5.77 per cent than that in the commercial hams. A similar relationship exists for all of the other subsamples except No. 3, the center subsample, in the case of which the commercial hams are higher in salt content by 1.33 per cent.

Somewhat better uniformity is apparent for all of the hams than was the case with the 75-degree cure, a fact perhaps due to the better penetration of the salt from the 85-degree brine. A further fact which may contribute to the uniformity of the data for this cure is that in some cases hams of this cure were subjected to a somewhat longer aging period than in the other cases during which there was more complete equalization of the salt throughout the meat tissue.

DISCUSSION OF RESULTS

A summary of the data with respect to the salt content of all of the hams used in this study (Table 5) shows that the average salt content of the entire center slice for all three groups of experimentally cured hams was about the same, and was somewhat lower than that of the commercial hams. Also, with respect to the various subsamples, on the average, none of the experimental hams was as salty as the commercial hams. There was no evidence that the lower salt content of these hams impaired their keeping quality. This does not mean that a somewhat higher salt content would not be desirable from the

TABLE 4

Salt Content of Hams Cured in 85-Degree Sweet Pickle

Ham No.	Curing period	Aging period	Curing temperature	Fresh weight		Thickness	Mean NaCl content [1] of center slice	NaCl content [1]			
								Sub-sample No. 2	Sub-sample No. 3	Sub-sample No. 4	Sub-sample No. 5
	days	*days*		*lb.*	*oz.*	*in.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
24	32	30	1.1 to 2.8°C. (34 to 37°F.)	11	0	$4\frac{2}{7}$	10.00	9.46	9.30	10.97	10.28
25	28	61	Uncontrolled	14	14	$5\frac{1}{2}$	8.02	7.32	8.99	8.24	7.52
26	35	54	Uncontrolled	15	5	$5\frac{1}{2}$	9.46	8.30	8.90	10.21	10.41
27	42	61	Uncontrolled	15	14	$5\frac{1}{2}$	11.15	9.76	13.08	11.12	10.64
28	28	30	1.1 to 2.8°C. (34 to 37°F.)	13	7	5	9.58	9.30	9.33	9.76	9.92
29	42	60	1.1 to 2.8°C. (34 to 37°F.)	16	10	$5\frac{1}{2}$	11.29	11.45	12.23	10.82	10.64
30	35	60	1.1 to 2.8°C. (34 to 37°F.)	14	3	5	9.63	11.32	10.61	9.10	7.49
31	35	30	1.1 to 2.8°C. (34 to 37°F.)	12	10	$4\frac{1}{7}$	13.29	10.39	10.02	15.91	16.84
32	28	30	1.1 to 2.8°C. (34 to 37°F.)	10	3	$3\frac{5}{7}$	9.48	9.16	6.63	11.84	10.29
Mean	....	....	....	....	....	.....	10.21 ± .34	9.61	9.90	10.89	10.45

[1] NaCl in dry matter.

standpoint of keeping quality under uncertain storage and handling conditions, but the keeping quality of these hams is judged to be satisfactory for farm conditions.

It will be observed that some of the hams were cured under controlled temperature conditions at 1.1 to 2.8°C. (34 to 37°F.) and that some were cured under uncontrolled conditions which were approximately 8.3°C. (47°F.) in the winter and 12.8°C. (55°F.) in the spring and fall. No differentiation has been made in this study between hams cured under these two different temperature conditions because they prevailed only during the curing period proper, and all of the hams were subsequently exposed to approximately the same environmental temperatures during the aging or holding period which followed the initial period in cure.

TABLE 5

Average Salt Content [1] of Center Slice of Hams

Number of hams	Kind of ham	Average salt content [1]				
		Entire center slice	Sub-sample No. 2	Sub-sample No. 3	Sub-sample No. 4	Sub-sample No. 5
		pct.	*pct.*	*pct.*	*pct.*	*pct.*
8	Commercial (purchased locally)...	14.59 ± .51	14.58	11.23	16.32	16.22
11	Sweet pickle (75°) cure................	10.15 ± .48	8.79	7.69	11.17	13.05
9	Sweet pickle (85°) cure................	10.21 ± .34	9.61	9.90	10.89	10.45
12	Dry-cured..	10.51 ± .38	9.64	9.16	10.80	12.44

[1] NaCl in dry matter.

Also, heretofore no mention has been made concerning the length of curing periods. The number of days in cure is given in the tables and will be found to be different for different hams. These curing periods were established for purposes other than the immediate object of this paper, and the chief justification for use of the data presented here is that the initial curing periods were followed by the above-mentioned aging or seasoning period which was at least 30 days long. During this period all of the hams were subjected to the same environmental conditions and were smoked. It was during this period that the cure was really completed. No further absorption of salt is possible during this time, but that which already has been absorbed, and is present to a large extent in most cases in the outer portions of the ham, continues to penetrate the tissue and to strike into the center of the ham, eventually becoming more or less evenly distributed throughout the whole piece. This process of sale equalization has previously been reported on by Ziegler and Miller (1938).

This latter consideration does not ignore the fact that in brine-curing more salt is taken up by the meat upon longer contact with the curing agent. However, there has been some misconception concerning the length of the curing period as applied to dry-curing. In this case, inasmuch as the curing agent is applied directly to the meat in definite amounts and is all absorbed within a relatively few days, the proper conception of the curing period is a length of time sufficient for the salt to strike into the meat and become distributed in concentrations sufficient to prevent spoilage. A prolonged curing period, in the case of dry-curing, will not affect the amount of salt ultimately present in the meat, this having been determined by the amount of curing agent that was applied.

In the case of the brine-cure a different situation exists and the length of the curing period directly influences the amount of salt taken up by the meat. The authors have thought it more logical to consider the length of the curing period in relation to the thickness of the cuts to be cured rather than in relation to their weight. This viewpoint is arrived at from a consideration that the efficacy of salt in curing is due to its penetration of the meat, a process most logically considered in relation to the distance through the meat that it is necessary for the salt to travel or, in other words, the thickness of the cut rather than its weight. The concentration of salt in the meat must be considered in relation to the weights of salt and meat; the length of time required to permit the salt to penetrate a given cut is proportional to the thickness of the meat.

On this basis the average length of cure for the hams cured with the 75-degree pickle was eight days per inch of thickness and for those with the 85-degree pickle, seven days per inch. Curing periods of this order have produced the amounts of salt in the cured hams as given in the tables. These hams appear to be on the average somewhat lower in salt content than the commercial hams studied. Further work by the authors to be reported elsewhere has indicated that curing periods of nine days per inch with an 85-degree pickle produce hams with a salinity approaching that of the commercial hams reported here.

SUMMARY

Data are presented concerning the salt content of 40 hams; of these eight were commercial hams purchased locally and the remainder were experimentally cured as follows:

(1) Twelve hams dry-cured with eight pounds of salt, three pounds of sugar, and three ounces of saltpeter applied at the rate of one ounce per pound of meat.

(2) Eleven hams brine-cured with a 75-degree sweet pickle made by dissolving the above 8-3-3 mixture in five gallons of water.

(3) Nine hams brine-cured with an 85-degree sweet pickle made by dissolving the 8-3-3 mixture in four gallons of water.

The analysis of samples taken from these hams is presented as well as the analysis of samples taken from three commercial brands of ham.

The average amount of salt present in the dry matter of the subsamples of the experimentally cured hams was practically the same for the three cures and averaged 10.29 per cent as compared with 14.59 per cent for the commercial hams, indicating that the methods used produced a milder cure under the conditions studied.

The relationship of the length of the curing period to the thickness of the ham is discussed in the case of brine-curing. Production of hams of higher salt content is possible by use of a longer curing period per inch of thickness of the meat than that used in this study.

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it is not these two vitamins but still another factor in yeast that is essential for the growth of *B. anthracis*.

The *B. anthracis* growth factor is not replaced by thiamin, nicotinic amide, beta alanine, pimelic acid, glutathione, betaine, riboflavin, glucosamine, vitamin B_6, or the acidic ether extract of yeast, either singly or in various combinations.

Meat extract, liver extract and yeast are good sources of the growth factor.

Two concentrates of the *B. anthracis* factor prepared from yeast exhibited growth stimulation at levels of 1 and 30 micrograms, respectively.

High vacuum distillation of a potent concentrate at temperatures from 120° to 160°C. at 0.002 mm. mercury results in the activity remaining in the residue.

The growth factor is resistant to autoclaving, to acid and alkali, and to light, is adsorbed on norite charcoal at pH 1.4, is soluble in water and aqueous alcohol, and is insoluble in acetone, ether and chloroform.

CENTRAL PENNSYLVANIA BRANCH

State College, Pa., November 26, 1938

Comparative Study of the Intracutaneous Injection of P.P.D. and the Vollmer Patch Test for the Detection of Tuberculosis. *John W. Rice*, Bucknell University.

Due to reluctance on the part of many college students to submit to the use of the hypodermic needle, it was decided to study the patch test devised by Vollmer for the testing of young children.

A group of 265 students were tested by intracutaneous injection of P.P.D. in standard dose and simultaneously with the Vollmer Patch Test. To summarize, 52.8% of the group were negative to both tests; 23% were negative to the Vollmer test but showed a one plus reaction to P.P.D.; 13.6% showed agreement in one plus reactions while in the group of fairly strong reactions to P.P.D., 0.75% were negative; 2.64% were faintly positive; and 1.6% were definitely positive to the Vollmer test. A total of 5.3% showed stronger reactions to the Vollmer test than to P.P.D. Delayed reactions were quite common in the case of the Vollmer test.

The relative high number of negative reactions together with the delayed response of the Vollmer Patch Test would seem to indicate that this test is not applicable to the college age group.

Studies of Cacao Fermentation. *S. Hoynak, T. S. Polansky and R. W. Stone*, Pennsylvania State College.

A study has been made of the microflora of cacao beans, together with some resulting chemical changes produced by the microorganisms during fermentation. Counts of viable organisms were made daily during the fermentation period. Relative percentages of types, the stage of their appearance in the fermentation, and their isolation and identification were carried out.

During the initial or alcoholic stage of the fermentation, yeast-like organisms predominate. This is followed by an acetic and later a proteolytic stage. In both these latter stages gram-negative rods are most numerous. Utilization of reducing sugars, pH, and titratable acidity were followed. Determinations were made on fermenting beans at 25°C., 36°C., and 45°C. Under the experimental conditions, variations in temperature generally resulted in a change of rate rather than in type of fermentation.

A Study of the Epidemiology of Tobacco Wildfire. *H. D. McAuliffe, M. A. Farrell, D. E. Haley and J. J. Reid,* The Pennsylvania Agricultural Experiment Station.

The epidemiology of tobacco wildfire has been the subject of a study carried out as a threefold program consisting of a general survey of the tobacco-growing regions of Pennsylvania, soil fertility experiments and laboratory investigations.

It has been found that the incidence of tobacco wildfire cannot be related to the incidence of *Phytomonas tabaca* inasmuch as the organism has been present in all tobacco examined, whether exhibiting lesions or free from all evidence of disease.

On the other hand, normal tobacco of the cigar-leaf filler type has been found to be very resistant to infection of economic severity during the growing season. Normal tobacco contains a nitrogen-potassium ratio of somewhat less than one. Tobacco with a ratio greater than one has been found relatively susceptible during the growing season.

During the ripening period, tobacco may become susceptible regardless of previous nutrition if any significant amount of nitrogen is taken up by the plant. This susceptibility has been found to be increased by topping and suckering, practices which take from the plant the last possibility of the normal use of nitrogen at this period in the proliferation of new tissue.

The conclusion is drawn that the control of tobacco wildfire is largely a matter of plant nutrition.

The Relation of Delayed Ammonification and Nitrification to Leaf Spots of Tobacco. *J. Naghski, H. D. McAuliffe, D. E. Haley and J. J. Reid,* The Pennsylvania Agricultural Experiment Station.

Susceptibility of tobacco to leaf spot diseases during the ripening period has been found to be associated with a significant nitrogen uptake at this stage.

The presence of nitrates in the soil in excessive amounts during the ripening period has been shown to be due to delayed ammonification and nitrification of the organic nitrogen applied in the spring in some instances, and in others to delayed nitrification of ammonium salts which have probably remained for extensive periods in some relatively insoluble form such as ammonium magnesium phosphate.

Delay in ammonification and nitrification is usually associated with lack of sufficient rainfall in the growing season. If this condition is followed by rain during the ripening period an excessive amount of ammonification and nitrification takes place, the normal resistance of tobacco to leaf spot diseases is lost and a serious outbreak of disease occurs.

The conclusion is drawn from these studies that it is unsafe to incorporate large quantities of complex organic nitrogen in a soil to be used for tobacco. From this standpoint tobacco should not follow a legume in the rotation nor should any animal manure be applied to the crop that has not been previously rotted.

A Cultural and Serological Study of Phytomonas tabaca and Certain Related Forms. *J. J. Reid, J. Naghski, R. G. Harris and H. D. McAuliffe,* The Pennsylvania Agricultural Experiment Station.

A study has been made of a number of strains of *Phytomonas tabaca* and strains of a few other species of the genus *Phytomonas* associated with leaf spot diseases. These have been investigated physiologically and serologically and compared with known strains of *Pseudomonas fluorescens* and with more than 2000 isolations of *P. fluorescens* from the leaves of normal, healthy tobacco and clover.

It has been found possible through culture in unheated plant extracts high in nitrogen to bring about a physiological adaptation of *Pseudomonas fluorescens* that is physiologically and serologically identical with strains of *Phytomonas tabaca.*

Such adaptation may be induced rapidly in the laboratory and may be as rapidly destroyed by frequent passage through other laboratory media.

As a result of this study the conclusion has been drawn that *Phytomonas tabaca* represents merely a transitory physiological adaptation of *Pseudomonas fluorescens.*

The work with the other representatives of *Phytomonas* associated with leaf spots while not as conclusive, suggests that other members of the genus are also temporary physiological adaptations of *Pseudomonas fluorescens.*

EASTERN MISSOURI BRANCH

November 8, 1938

Studies on Rabies. 1. Comparison of Guinea Pig and Mouse Inoculation Methods. *S. Edward Sulkin and Joseph C. Willett,* Laboratories of the St. Louis Health Division, St. Louis, Missouri.

A simple technique for the early diagnosis of rabies by mouse inoculation is described as a convenient means of verifying the routine microscopic (Negri body) method. The results of the experiments presented show that (1) the white mouse is the animal of choice in the laboratory diagnosis of rabies, Swiss mice being of no practical advantage; (2) that the demonstration of Negri bodies in the test animal is the only dependable criterion for a positive diagnosis since clinical manifestations are frequently too variable and indefinite to be of any value; (3) that it is practical to attempt demonstration of Negri bodies by sacrificing mice on the 8th or 9th day after the inoculation; and (4) that the mouse inoculation test offers a more expedient and cheaper means of diagnosis than the guinea pig method.

Studies on Rabies. 2. Demonstration of Rabies Virus in Grossly Decomposed Animal Brains. *S. Edward Sulkin and Nathan Nagle,* Laboratories of the St. Louis Health Division, St. Louis, Missouri.

Putrified brain tissue is usually difficult material in which to demonstrate Negri bodies and for this reason the diagnosis of a decomposed brain by microscopic examination is unreliable.

Experiments are presented describing a simple procedure for demonstrating rabies virus in grossly decomposed animal brains. In the preliminary experiments several bactericidal agents including merthiolate, glycerol, phenol, and ether were used in an effort to find one which would conveniently sterilize the heavily infected inoculum without appreciably effecting the virus. Ether used according to the method suggested by Dr. John R. Paul (J. Bact., *35,* 493,

1938) and others proved to be the most desirable agent. It was found that ether in final concentration of 10% exerts a definite bactericidal effect upon contaminated dog brains after exposure for 2 hours at 4°C. Exposure to this concentration of ether for 18 hours at 4°C. had no effect upon the virulence of two strains of street virus tested. The experiments were conducted with the view of establishing a simple and practical method for treating contaminated brains before intracerebral injection into animals.

PROPHYLAXIS OF TETANUS. *Philip Varney*, Department of Bacteriology, School of Medicine, Washington University, St. Louis.

REPORT ON THE HOT SPRINGS, ARKANSAS, CONFERENCE ON SYPHILIS. *R. B. H. Gradwohl*, Gradwohl Laboratories, St. Louis.

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FLAVOR OF TURKEY MEAT AS AFFECTED BY FEEDING FISHMEAL AND FISH OIL[1]

By R. R. Murphy, *Assistant Professor of Poultry Husbandry,* R. V. Boucher, *Associate Professor of Agricultural and Biological Chemistry, and* H. C. Knandel, *Professor of Poultry Husbandry, The Pennsylvania State College, State College, Pennsylvania, U. S. A.*[2]

INTRODUCTION

Under some conditions of feeding and management, feeds of marine origin when fed to turkeys impart a definite flavor of fish to the meat. This fishy flavor in turkey carcasses has caused extensive loss to producers by the unfavorable reaction created among consumers. In order to avoid the production of turkey meat having a fishy flavor, many growers have discontinued the use of fish oil and fishmeal in the ration without realizing the extent of the loss of valuable proteins, minerals, and vitamins. Many byproduct feeds of the fish industry are important constituents of the ration since they supply a considerable portion of the necessary nutrients for satisfactory growth and bone development.

Only a limited amount of information has appeared in the literature concerning the problem, even though it is one of economic importance among turkey producers. Carrick and Hauge[3] reported that cod-liver oil, when fed in sufficient quantity, imparted a fishy taste to the flesh of chickens. A press release of the United States Department of Agriculture in December 1936 indicated that fishmeal and cod-liver oil, when fed separately or in combination, gave a fishy flavor to the meat of chickens and turkeys. Cosby and Knowlton[4] of the Oregon Agricultural Experiment Station have shown that certain grades and combinations of fishmeals and vitamin D oils when fed to turkeys will produce fishy or off-flavored carcasses when cooked.

Marble, Hunter, Knandel, and Dutcher[5] observed a fishy flavor and odor in the roasted carcasses of turkeys 28 weeks of age fed either 1 percent of a poultry-grade cod-liver oil (not U.S.P.) or 10 percent of vacuum-dried white fish meal, or both, during the entire growth period. A mash mixture containing these two ingredients when fed to turkeys produced a more pronounced flavor and odor than when fed separately.

These findings are not in accord with those of Asmundson and associates,[6] which indicate that a ration containing 25 percent of a high-grade fishmeal when fed to turkeys for 6 or more weeks prior to slaughter apparently did not produce an off-flavor in the meat. However, off-flavors and odors were observed in the carcasses of birds fed either 2 or 5 percent of fish oil and 25 percent of fishmeal.

In the work of Marble and coworkers[5] birds were removed from the groups fed either cod-liver oil or fishmeal or a combination of both, at 2, 4, and 8 weeks prior to slaughter and placed on a basal diet containing neither cod-liver oil nor fishmeal. Removal of both cod-liver oil and fishmeal from the diet 8 weeks before slaughter practically eliminated the occurrence of both fishy flavor and odor.

The object of the work here reported was to study the effect of various fish products used in turkey rations on the flavor of the meat produced, and to determine which products could be retained in the ration with minimum risk of imparting objectionable flavors and odors to the meat.

EXPERIMENTAL PROCEDURE

Day-old Bronze turkey poults hatched July 13, 1937, were fed for a period of 8 weeks an all-mash, 24-percent protein starting diet consisting of 28 pounds of ground yellow corn, 10 pounds of wheat bran, 10 pounds of standard wheat middlings, 10 pounds of ground heavy oats, 5 pounds of alfalfa leaf meal, 10 pounds of dry skim milk, 15 pounds of meat scrap, 10 pounds of white fishmeal, 1 pound of salt, and 1 pound of cod-liver oil.

At 8 weeks of age the poults were divided into six groups of 12 poults each. Each group was housed in a 10 by 12 foot colony brooder house with access at all times to a wire-covered sunporch. The houses were identically constructed and equipped. An all-mash diet containing approximately 20 percent of protein was fed from the eighth to the twenty-eighth week (table 1). The diet for each group was modified as follows:

Table 1.—*Composition of experimental diets*

Ingredient	Group No.—					
	1	2	3	4	5	6
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Ground yellow corn	31	30	30¾	30¾	31	31
Wheat bran	15	15	15	15	15	15
Standard wheat middlings	15	15	15	15	15	15
Ground heavy oats	10	10	10	10	10	10
Alfalfa leaf meal	5	5	5	5	5	5
Dry skim milk	5	5	5	5	5	5
Meat scrap	17	17	17	17	7	7
Ground limestone	1	1	1	1	1	1
Salt	1	1	1	1	1	1
Cod-liver oil (85 units of vitamin D)	0	1	0	0	0	0
Sardine oil (400 units of vitamin D)	0	0	¼	0	0	0
Cod-liver oil (400 units of vitamin D)	0	0	0	¼	0	0
Vacuum-dried white fish meal	0	0	0	0	10	0
Menhaden fish meal	0	0	0	0	0	10
Total	100	100	100	100	100	100

Group No.	Modifications in basal ration
1	None.
2	1 percent of a satisfactory grade of cod-liver oil, commonly used for animal-feeding, assaying 85 Association of Official Agricultural Chemists chick units of vitamin D per gram in place of 1 percent of ground yellow corn.
3	0.25 percent of sardine oil assaying 400 Association of Official Agricultural Chemists chick units of vitamin D per gram in place of 0.25 percent of ground yellow corn.
4	0.25 percent of cod-liver oil assaying 400 Association of Official Agricultural Chemists chick units of vitamin D per gram in place of 0.25 percent of ground yellow corn.
5	10 percent of vacuum-dried white fishmeal in place of 10 percent of meat scrap.
6	10 percent of menhaden (dark) fishmeal in place of 10 percent of meat scrap.

At 28 weeks of age, one male and one female from each of the 6 groups, or 12 birds in all, were starved for a period of 24 hours and then slaughtered, dry-picked, drawn, and quick frozen. The carcasses were held in a frozen condition for a period of 2 weeks. Each of 12 carcasses was then removed and roasted in a separate oven at a temperature of 325° F. No filling or seasoning was used. During the roasting period each turkey was frequently basted in its own juice. The giblets of each bird were boiled in separate containers.

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[1] Authorized for publication on September 23, 1938, as paper No. 853 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] The authors wish to acknowledge the assistance of the Home Economics Department of The Pennsylvania State College in roasting the turkeys and in scoring the carcasses.

[3] Carrick, C. W., and Hauge, S. M. The effect of cod-liver oil upon flavor in poultry meat. Poultry Sci. 5:213-215. 1926.

[4] Cosby, H. E., and Knowlton, F. L. Third Progress Report on Fishy Flavored Turkeys. Oreg. Agr. Expt. Sta. (Unpublished data.)

[5] Marble, D. R., Hunter, J. E., Knandel, H. C., and Dutcher, R. A. Fishy flavor and odor in turkey meat. Poultry Sci. 17:49-53. 1938.

[6] Asmundson, V. S., Jukes, T. H., Fyler, Harriet M., and Maxwell, Margaret L. The effect of certain fish meals and fish oils in the ration on the flavor of the turkey. Poultry Sci. 17:147-151. 1938.

On removal from the oven, each carcass was scored individually for fishy flavor and odor by 20 persons. The identity of the carcasses was not disclosed to those who assisted in their preparation nor to those who participated in the scoring. The tasters did not discuss their observations with one another until after the scoring had been completed. Odor of each carcass and flavor of the breast meat, thigh meat, giblets, and drippings were observed in the order named. Each person indicated the degree of flavor and odor of fish as none, slight, medium, or strong.

In place of a fork, a new toothpick was used each time a piece of meat was tasted. Clean glass stirring rods were used for tasting the drippings. To destroy the flavor between samples tasted, each person ate a small piece of dry bread and drank black coffee before proceeding to the next sample. A new paper cup was used for each serving of coffee. Every precaution was taken to avoid the possibility of the flavor of one sample influencing the flavor of another. Since, in previous work, a close correlation existed between the scorings of hot and cold meat, all carcasses were scored only while the meat was hot.

RESULTS AND DISCUSSION

It is realized that taste and smell are senses which are very difficult to measure quantitatively. Variation was found to exist in the taste reaction of different individuals. In order to overcome this variation as many tasters as feasible were used. Imagination seemed to influence the decision of some tasters, who reported fishy flavor and odor in carcasses of turkeys fed the basal diet containing neither fishmeal nor oil. Others did not detect either the flavor or odor of fish in samples in which both were very apparent to most tasters. However, the scores of all individuals who participated in the scoring of the carcasses are included in tables 2 and 3.

A greater degree of fishy flavor was observed in the dark meat than in the white meat. In the drippings the fishy flavor was more pronounced than in the meat. This was true for all five groups in which a substitution had been made in the basal diet. These observations are in accord with those of Marble and associates.[7]

The distribution of scores recorded by 20 observers on the flavor of the breast meat, thigh meat, giblets, and drippings is shown in table 2. Table 3 presents the distribution of scores on the odor of the entire carcass. One percent of cod-liver oil of a grade commonly used for animal feeding, when fed in a ration without fishmeal to turkeys for a period of 20 weeks previous to time of slaughtering, imparted to the meat a definite fishy flavor and odor. One-fourth of 1 percent of a high-potency vitamin D oil was fed in a ration without fishmeal to each of two groups of turkeys. These high-potency oils supplied to the ration practically the same number of units of vitamin D as 1 percent of the 85 unit cod-liver oil. The carcasses of turkeys fed one-fourth of 1 percent of high-potency oil were practically free from any flavor or odor of fish.

A slight flavor and odor of fish were detected in the carcasses of turkeys fed a ration containing either 10 percent of menhaden (dark) fishmeal or 10 percent of vacuum-dried white fishmeal. Ten percent of menhaden fishmeal in a ration without fish oil seemed to impart a slightly stronger flavor and odor than vacuum-dried white fishmeal.

TABLE 2.—*Distribution of scores made by 20 observers for fishy flavor in the cooked meat of 28-week-old turkeys*

Breast meat

Substitutions in basal ration[1]	Scores for female turkeys showing indicated degree of fishy flavor				Scores for male turkeys showing indicated degree of fishy flavor			
	None	Slight	Medium	Strong	None	Slight	Medium	Strong
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
None	20	0	0	0	20	0	0	0
1 percent cod-liver oil	11	5	4	0	16	3	0	1
0.25 percent sardine oil	20	0	0	0	19	1	0	0
0.25 percent cod-liver oil	20	0	0	0	18	2	0	0
10 percent white fishmeal	18	2	0	0	19	1	0	0
10 percent menhaden fishmeal	18	1	1	0	20	0	0	0
Thigh meat								
None	20	0	0	0	19	1	0	0
1 percent cod-liver oil	7	4	6	3	13	5	1	1
0.25 percent sardine oil	19	1	0	0	15	5	0	0
0.25 percent cod-liver oil	19	1	0	0	15	5	0	0
10 percent white fishmeal	15	3	2	0	15	5	0	0
10 percent menhaden fishmeal	17	2	1	0	15	4	1	0
Giblets								
None	20	0	0	0	17	3	0	0
1 percent cod-liver oil	10	5	2	3	16	2	2	0
0.25 percent sardine oil	18	2	0	0	16	3	1	0
0.25 percent cod-liver oil	19	1	0	0	15	4	0	1
10 percent white fishmeal	15	3	2	0	15	5	0	0
10 percent menhaden fishmeal	12	7	1	0	15	3	2	0
Drippings								
None	19	1	0	0	14	5	1	0
1 percent cod-liver oil	3	3	6	8	9	4	5	2
0.25 percent sardine oil	15	3	2	0	14	3	3	0
0.25 percent cod-liver oil	19	1	0	0	11	6	3	0
10 percent white fishmeal	10	6	3	1	11	4	3	2
10 percent menhaden fishmeal	9	7	4	0	8	6	5	1

[1] Fed for a period of 20 weeks before slaughtering.

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[7] See footnote 5.

Carcasses of turkeys fed 1 percent of cod-liver oil exhibited a more pronounced fishy flavor and odor than did those fed 10 percent of either white or dark fishmeal.

At 28 weeks of age all turkeys on experiment were examined and the conformation of the breastbone noted. The breastbone of each bird was classified as straight or as one of three arbitrarily chosen degrees of deformity, namely, very deformed, deformed, and slightly deformed. Table 4 gives a summary of these observations. From a limited number of observations there was evidence to indicate that the retention of a source of vitamin D in the ration during the period of growth had a beneficial effect on the conformation of the breastbone.

TABLE 3.—*Distribution of scores made by 20 observers for fishy odor in the cooked meat of 28-week-old turkeys*

Substitutions in basal ration[1]	Scores for female turkeys showing indicated degree of fishy odor				Scores for male turkeys showing indicated degree of fishy odor			
	None	Slight	Medium	Strong	None	Slight	Medium	Strong
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
None	20	0	0	0	13	7	0	0
1 percent cod-liver oil	1	7	6	6	10	4	5	1
0.25 percent sardine oil	13	7	0	0	14	4	2	0
0.25 percent cod-liver oil	19	1	0	0	10	7	3	0
10 percent white fishmeal	9	9	2	0	12	7	1	0
10 percent menhaden fishmeal	8	10	2	0	9	9	1	1

[1] Fed for a period of 20 weeks before slaughtering.

TABLE 4.—*Effect of vitamin D in the ration on conformation of breastbone of 69 turkeys 28 weeks of age*

Groups	Turkeys having indicated breastbone conformation			
	Straight	Slightly deformed	Deformed	Very deformed
	Number	*Number*	*Number*	*Number*
Fed vitamin D:				
No. 2	10	2	0	0
No. 3	11	1	0	0
No. 4	7	1	1	2
Total	28	4	1	2
Fed no vitamin D:				
No. 1	6	4	0	1
No. 5	7	0	1	3
No. 6	7	2	1	2
Total	20	6	2	6

SUMMARY

Under some conditions of feeding and management, feeds of marine origin when fed to turkeys impart a definite flavor of fish to the meat. This fishy flavor in turkey carcasses has caused extensive loss to producers by the unfavorable reaction created among consumers. In order to avoid the production of turkey meat having a fishy flavor, many growers have discontinued the use of fish oil and fishmeal in the ration without realizing the extent of the loss of valuable proteins, minerals, and vitamins. Many byproduct feeds of the fish industry are important constituents of the ration since they supply a considerable portion of the necessary nutrients for satisfactory growth and bone development.

The object of the work here reported was to study the effect of various fish products used in turkey rations on the flavor of the meat produced and to determine which products could be retained in the ration as sources of protein, minerals, and vitamins with minimum risk of imparting objectionable flavors and odors to the meat.

Twelve carcasses of turkeys fed different fish products for varying periods of time were roasted in separate ovens. The degree of fishy flavor in the breast meat, thigh meat, giblets, and drippings was determined by 20 observers. The degree of fishy odor also was determined for all carcasses.

Carcasses of turkeys 28 weeks of age which had received a ration containing 1 percent of cod-liver oil but no fishmeal for a period of 20 weeks prior to slaughter exhibited a pronounced fishy flavor and odor when cooked. When 0.25 percent of either of two high-potency vitamin D oils was fed in a ration without fishmeal to turkeys of the same age and for the same period of time, carcasses were produced which when roasted were practically free from any flavor or odor of fish.

Evidence was obtained which indicated that a slight flavor and odor of fish were present in the carcasses of turkeys fed a ration containing either 10 percent of menhaden (dark) fishmeal or 10 percent of vacuum-dried white fishmeal. Ten percent of menhaden fishmeal appeared to impart a slightly stronger fishy flavor and odor than vacuum-dried white fishmeal. Carcasses of turkeys fed 1 percent of cod-liver oil exhibited a more pronounced flavor and odor than did those fed 10 percent of either white or dark fishmeal.

Limited evidence was obtained which indicated that retention of a source of vitamin D in the ration throughout the growing period influenced favorably the production of straight breastbones.

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10344

Comparative Nutritive Value of Firm and Watery Egg Albumen.*

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From the Pennsylvania State College.

The question of egg quality has become a matter of economic concern to the producer, the distributor and to the consumer. Investigators who have attempted to study this problem have been unable to agree as to what constitutes egg quality. Needless to say this lack of agreement has been a considerable handicap to researches along this line. However, in spite of this lack of agreement as to the characteristics of quality, eggs with excessive amounts of watery white have been discriminated against by the consumer and also by the wholesale distributor, mainly because the former has found that such eggs present a less desirable appearance when poached or fried than do eggs having the firm white. In contrast to this particular objection, some researches have shown that the watery egg white has more desirable whipping qualities than does the firm white. While watery and firm albumen differ markedly in physical characteristics, no data are available to show that they differ in nutritive properties.

Because of the increasing interest in the subject of egg quality, it appeared to us that an effort should be made to determine whether the physical state of the albumen (watery or firm) of a freshly laid egg is an index of nutritive value. With this point in view, a series of studies were made to determine the nutritive value of the two types of albumen as indicated by their relative capacity to produce growth in healthy young rats.

The eggs used in these studies were obtained from hens that were receiving an all-mash ration. The eggs were gathered and their fractionation carried out on the same day that they were laid. The method employed in fractionating the eggs was as follows: After breaking the shell, the total albumen was carefully separated from the yolk and poured on a brass sieve (openings 2.14 mm in diameter) while the yolk itself was placed in a separate glass receptacle. That portion of the albumen which passed through screen was designated as the watery albumen while that retained by the screen was likewise designated as firm albumen.

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After all eggs had been fractionated in this manner, each of the 3 portions, *viz.,* yolks, watery albumens, and firm albumens, was thoroughly mixed and weighed in order to determine its relative percentage in the original eggs. A weighed portion of the yolk and of the watery albumen was recombined so as to give a mixture having the same relative composition as the original eggs. A similar mixture was made using weighed portions of the yolk and firm albumen. Each mixture of yolk and albumen was then coagulated by heating in a double boiler, broken into small lumps and dried on cheesecloth in a current of warm air. The dried product was mixed, ground to a satisfactory degree of fineness and stored in an electric refrigerator until fed to the test animals. For the first nutritional test the eggs were prepared in small weekly batches, while for the second test sufficient eggs were prepared at the beginning of the experiment to last during the entire period.

The nutritional studies consisted in feeding carefully matched groups of healthy young rats, maintained in individual metal cages, a diet consisting of one of the egg combinations. In order to bring about a more favorable mineral balance calcium carbonate was fed *ad lib.* It was believed that differences in the nutritive value of the 2 types of albumen would be more readily demonstrated if the test diet consisted entirely of the egg products. The amount of egg fed daily was restricted so that each test animal consumed the same amount of the dried egg during the weekly period. The amount of diet was increased from week to week in order to compensate for increases in body weight of the animals. A liberal amount of calcium carbonate (U.S.P. grade) was maintained in a separate container in each cage. Each animal was weighed at weekly intervals and was given the usual care and attention accorded experimental animals. The first feeding test was continued for a period of 10 weeks while the second test lasted 12 weeks. It was believed that experiments of this duration were sufficient to determine whether or not marked differences existed in the nutritive value of the two types of albumen.

The growth responses made by these groups of animals are presented in the accompanying graph.

Curves A and B represent the average growth response of the test animals used in the first feeding test, while curves C and D represent similar data for the animals used in the second test. The

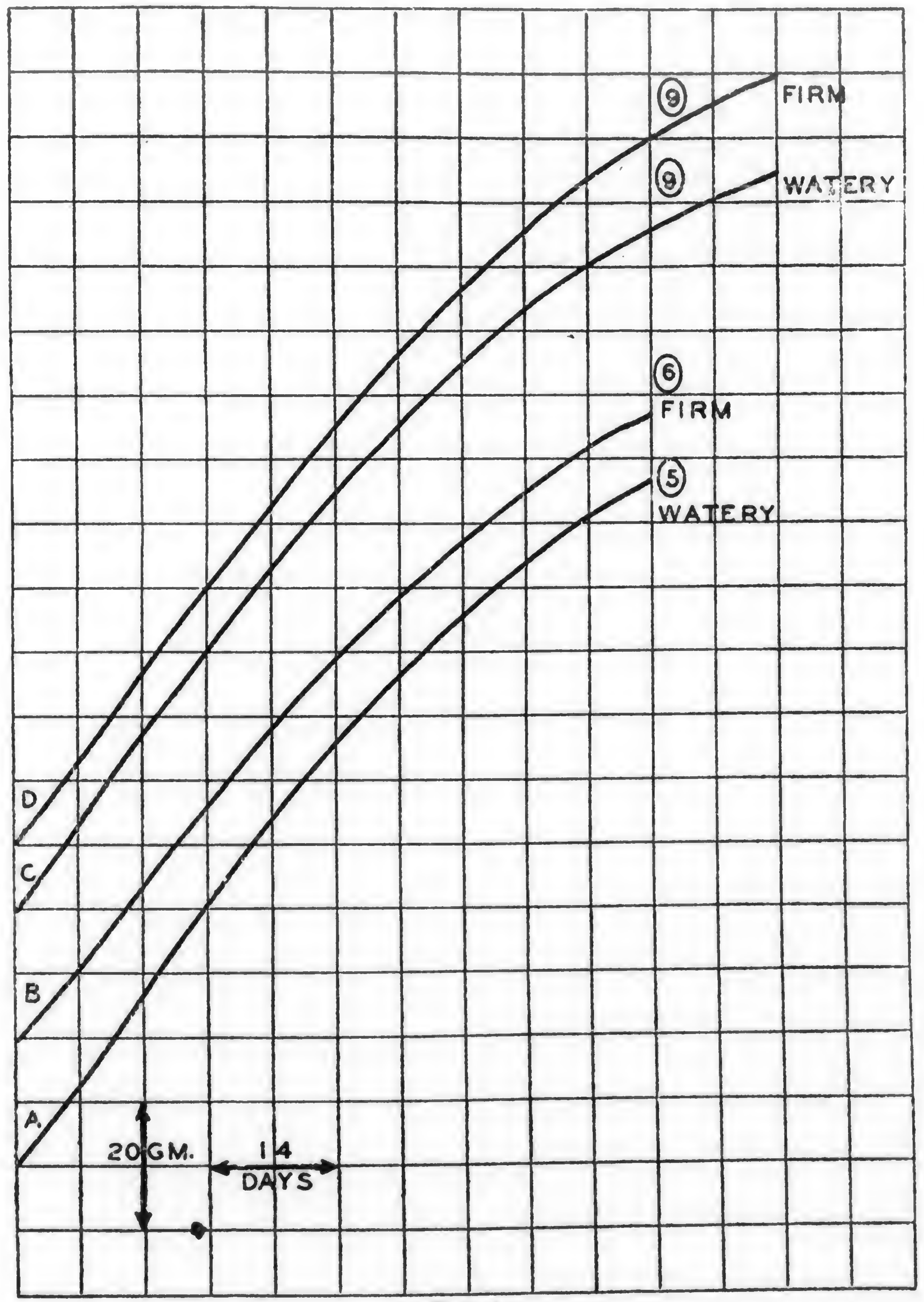

Fig. 1.

Average growth responses of young rats receiving diets composed entirely of dried eggs. These diets differd only in the type of albumen which they contained.

enclosed numerals denote the number of test animals considered in each feeding test.

It may be observed from the above graph that the growth responses made by the animals receiving the diets containing the 2 types of albumen are quite similar for both feeding trials. In the first test (Curves A and B) the animals which received the watery albumen made slightly greater gains in body weight than did their litter mates which received a similar diet containing firm albumen. The growth response made by the test animals used in the second feeding test (Curves C and D) was of approximately the same magnitude as that of the first test, but in this trial the animals receiving the diet containing the firm albumen made the greatest average gain. The average gains for the 12-week period were 119 and 115 g, respectively.

When the above growth responses were considered as a whole, from the standpoint of growth production per unit weight of moisture-free diet consumed, it was found that one gram of the diet containing the firm albumen produced an average gain in bodyweight of 0.2817 g, while an equal weight of the diet containing the watery albumen produced a gain of 0.2813 g. Such differences in growth responses are not significant. It, therefore, appears from the data obtained in the above feeding tests that there is no difference in the nutritive value of the 2 types of egg albumen.

Summary. Groups of young rats were fed diets composed solely of dried egg products, the difference in the diets being the type of albumen which they contained. The resultant growth of these animals indicates that there is no difference in nutritive value of watery and firm albumen.

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THE QUANTITATIVE DETERMINATION OF VITAMIN C IN MILK

Recent studies[1, 2, 3, 4] have shown that there are a number of important factors which may influence the results obtained in the determination of vitamin C in milk by titration with 2,6-dichlorobenzenoneindophenol. Such variables as temperature, light, method of standardizing the dye (2,6-dichlorobenzenoneindophenol), stability of the dye, contact of the milk with oxygen, catalytic elements present and methods of precipitating milk protein have been outstanding in these reports.

The present work was undertaken to simplify and to make reproducible a quantitative procedure for determining the total amount of vitamin C in milk as it comes from the dairy cow.

Kon and Watson[5] have shown that ascorbic acid (vitamin C) in milk as it leaves the cow's teat is in the reduced form. In this form the vitamin C content of milk may be determined quantitatively by direct titration with 2,6-dichlorobenzenoneindophenol. Should the milk, however, in the process of collection, be exposed even very briefly to contact with air, catalytic metals, light or heat, an unavoidable loss of ascorbic acid occurs due to oxidation. In order to eliminate the effect of these factors in collecting samples directly from cows of the college herd, a special apparatus was devised (See Fig. 1).

A 500 ml dark-glass bottle (A) was placed inside a 2-liter beaker and surrounded with ice and water. This was done a short time prior to milking in order that the bottle might become thoroughly chilled. The

[1] P. F. Sharp, *Jour. Dairy Science*, 21: 85, 1938.

[2] S. K. Kon and M. B. Watson, *Jour. Soc. Chem. Ind.*, 55: 508, 1936.

[3] R. R. Musulin and C. G. King, *Jour. Biol. Chem.*, 116: 409, 1936.

[4] C. H. Whitnah, W. H. Riddell and W. J. Caulfield, *Jour. Dairy Science*, 19: 373, 1936.

[5] S. K. Kon and M. B. Watson, *Biochem. Jour.*, 31: 223, 1937.

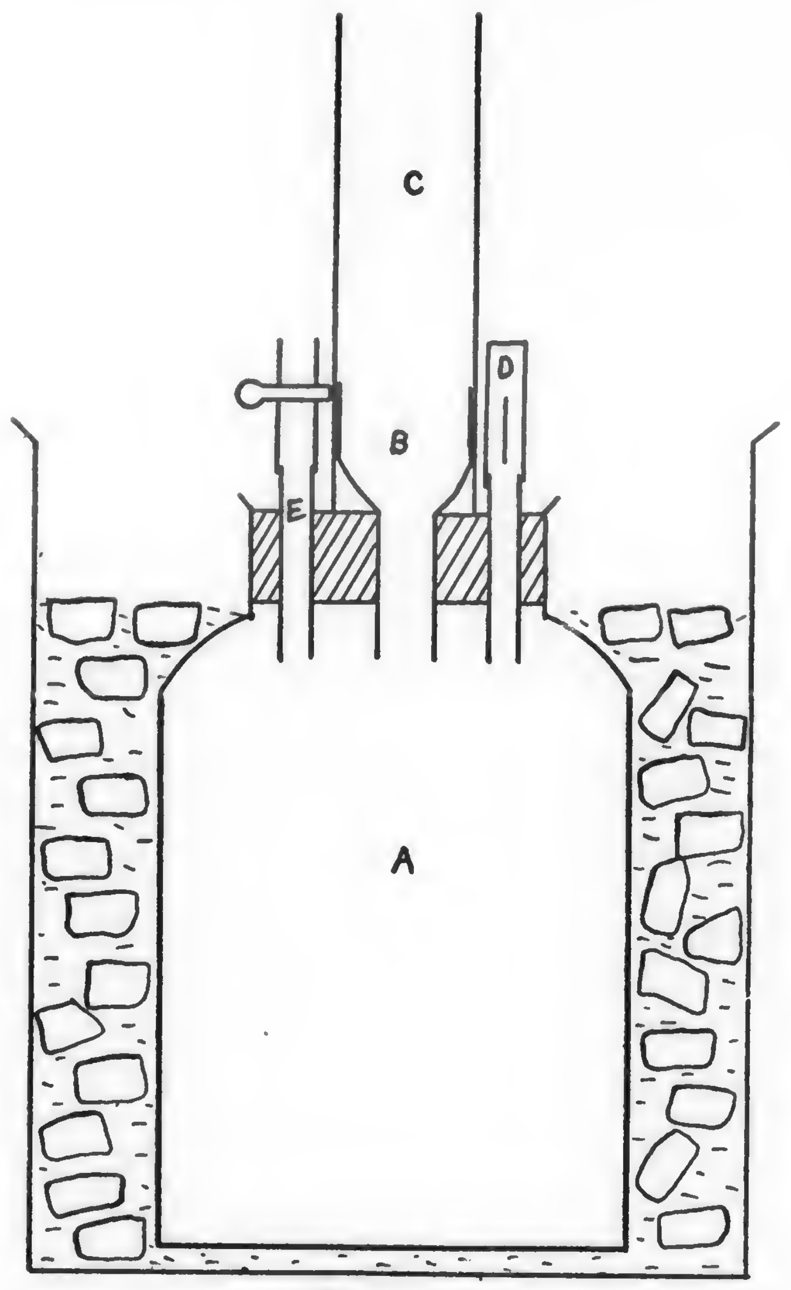

FIG. 1

bottle was fitted with a 3-holed rubber stopper. Through one hole of this stopper projected a glass adapter (B) having a funnel stem. A dark rubber tube (C), thin enough to be quite pliable and about one inch in diameter, was stretched over the adapter and pulled down to the rubber stopper. The upper part of this tube extended about three inches above the top of the adapter and was the part which was slipped over the teat of the cow during milking. A tube fitted with a Bunsen valve (D) passed through another hole in the stopper. Another glass tube (E) passed through the third hole and was provided with a short length of rubber tubing so that it could be closed off with a pinch clamp. The size of this tube was such as to permit the insertion of a 10 ml bulb pipette for removal of samples. Before milking, the air in the dark bottle was expelled through the Bunsen valve by closing all outlets with pinch clamps and flushing with carbon dioxide from a pressure tank.

To determine if the ascorbic acid of milk obtained in the above apparatus was completely in the reduced form, two samples were removed and titrated immediately, while other samples were subjected to treatment with hydrogen sulfide and then titrated after removing excess hydrogen sulfide with carbon dioxide. Typical results are given in Tables 1 and 2.

TABLE 1
TITRATION OF UNTREATED MILK SAMPLES

Ml milk	Ml dye	Mg ascorbic acid per aliquot	Mg ascorbic acid per liter
10.00	1.90	0.2280	22.80
10.00	1.91	0.2292	22.92
	Average . .	0.2286	22.86

TABLE 2
TITRATION OF HYDROGEN SULFIDE TREATED SAMPLES

Ml milk	Ml dye	Length of treatment in minutes	Mg ascorbic acid per aliquot	Mg ascorbic acid per liter
10.00	1.91	10	0.2292	22.92
10.00	1.91	10	0.2292	22.92
		Average	0.2292	22.92
10.00	1.92	30	0.2304	23.04
10.00	1.91	30	0.2292	22.92
		Average	0.2298	22.98
10.00	1.91	100	0.2292	22.92

The instability of 2,6-dichlorobenzenoneindophenol solutions reported by some workers was satisfactorily overcome by preliminary extraction of the dye with anhydrous ethyl ether. Solutions of dye, thus purified, and standardized according to the methods of Menaker and Guerrant,[6] remained perfectly stable and gave sharp end points over a period of 21 days.

Metaphosphoric acid was found by Fujita and Iwatake,[7] Lyman, Schultze and King[8] and others to protect vitamin C in solution against oxidation. This property, combined with the protein-precipitating ability of metaphosphoric acid, was made use of in the present work in preparing milk samples for titration. To eliminate the uncertain protein-precipitating power encountered with ordinary metaphosphoric acid solutions, the required volume of a stable sodium metaphosphate solution, prepared by the method of Briggs,[9] was acidified just before each titration and added to the milk. Using 10 ml of 10 per cent. sodium metaphosphate solution, the addition of 0.6 ml of concentrated hydrochloric acid was found to bring the pH of the solution to a point (pH 2.5–3.0) where immediate and complete flocculation of protein resulted upon addition of the metaphosphate solution to 10 ml of milk.

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[6] M. H. Menaker and N. B. Guerrant, *Jour. Ind. Eng. Chem.*, Anal. Ed., 10: 25, 1938.

[7] A. Fujita and D. Iwatake, *Biochem. Zeits.*, 277: 293, 1935.

[8] C. M. Lyman, M. O. Schultze and C. G. King, *Jour. Biol. Chem.*, 118: 757, 1937.

Titrations were made in the presence of precipitated milk protein. By repeated centrifuging and washing of the milk protein followed by separate titrations of combined centrifugate and protein residue, it was found that slightly higher ascorbic acid values were obtained in titrations of milk in the presence of precipitated protein. However, upon the addition of pure ascorbic acid to the protein residues and repetition of the centrifuging procedures and titrations, it was shown that the slightly higher apparent values obtained in the presence of milk protein were due to adsorption of negatively charged dye by positively charged protein. No adsorption of ascorbic acid by the milk protein could be demonstrated.

The new apparatus and improved technique provides a simple but reliable method of obtaining and determining vitamin C of milk in its naturally occurring form. The improvements mentioned should be of value in following fluctuations of vitamin C in milk at different stages of lactation, at various seasons of the years, during feeding experiments and under numerous other conditions.

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[9] D. Briggs, *Proc. Soc. Exp. Biol. and Med.*, 37: 634, 1938.

Problems of Feeding Cigarleaf Tobacco

By D. E. HALEY

Pennsylvania State College of Agriculture, State
College, Pennsylvania

Reprinted from
BETTER CROPS WITH PLANT FOOD MAGAZINE

This is typical of the fine buildings in the main tobacco districts of Lancaster County, Pennsylvania.

Problems of Feeding Cigarleaf Tobacco

By D. E. Haley

Pennsylvania State College of Agriculture, State College, Pennsylvania

THE problems involved in the production of cigarleaf tobacco in Lancaster County, the center of the tobacco industry of Pennsylvania, are many and varied. Those who are primarily interested in fundamental studies pertaining to this particular crop fully realize that any recommendations they may care to make, and especially those which involve a marked change in the agricultural practices commonly employed in the handling of this crop, must be made in the direct interest of the grower, the manufacturer, and the consumer.

The average tobacco grower of this locality produces his tobacco on soils which for the most part have been under cultivation for 200 years. In certain instances he has changed his practices as a matter of convenience. At other times he has accepted certain recommendations from State and Federal representatives which have been based on sufficient experimental evidence. In large measure, however, the methods he employs in producing tobacco are based primarily on personal experience and observations. He is open-minded, however, and will make certain fundamental changes if he fully realizes that it is to his interest to do so. In this he is aided materially by the advice of the local County Agent, Floyd S. Bucher, an experienced tobacco grower himself and one who is in close touch with those engaged in tobacco research.

The consumer demands among other things a cigar that burns well and which appeals to him from the standpoint of taste and aroma. The manufacturers have endeavored to standardize their product to meet with the approval of the consumer and with a remarkable degree of success, when it is considered that the tobacco they use may have been produced under widely different environmental conditions. However, the quality of their final product is closely associated with the quality of the original leaf itself.

Use of Nitrogen and Potash

The inherent qualities of the leaf, sought for by the manufacturer and consumer, depend primarily upon the way it has been handled on the farm. The problems relating to production, therefore, become of paramount importance. These involve, among many other things, the rational use of nitrogen and potash as fertilizing materials.

No attempt is made to minimize the importance of nitrogen in the nutrition of cigarleaf tobacco. An attempt is made, however, to call attention to the possible danger of adding an excess and to stress the importance of ready availability.

Nitrogen is absorbed and utilized normally by the growing plant in the early stages of growth, provided that it is supplied in a readily available form.

A good field of cigarleaf tobacco i

indicated by characteristic symptoms of potash deficiency during the growing season. In these cases a marked increase in yield may be noted when this particular fertilizer is applied. Of far greater importance, however, is the effect of potash on quality.

When a limited quantity of available potash is present, this tends to become localized in the upper portions of the tobacco plant. If the proper quantity is available, there tends to be a more uniform distribution of this material throughout the plant.

How Potash Affects Quality

It is a comparatively easy matter to demonstrate to all concerned that there is more or less of a definite relationship between the potash content of the plant and "wrapper" production, other things being equal. As the grower obtains far more financial returns from "wrappers" than from a lesser grade known as "fillers," this becomes a factor which he cannot well ignore.

It is a matter of common knowledge that a high potash content in the leaves reduces transpiration and therefore results in a better economy of the moisture supply. This is in part due to the formation of "cutin" which is found on the surface of the leaves, a factor of great importance during a drouth, as it interferes with the passage of moisture from the leaf to the atmosphere.

No element plays a greater role in the burning quality of tobacco than does potassium. Moreover, this element, in combination with malic and citric acids, appears to furnish a considerable amount of the energy necessary for the bacteria normal to the fermentation process.

The maximum benefits of a potash application are governed to a considerable extent by a number of environmental factors. The soil should be slightly acid, should contain a minimum of nitrogen organically combined, and should be properly balanced with respect to the other necessary fertilizer ingredients.

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No attempt is made to minimize the importance of nitrogen in the nutrition of cigarleaf tobacco. An attempt is made, however, to call attention to the possible danger of adding an excess and to stress the importance of ready availability.

Nitrogen is absorbed and utilized normally by the growing plant in the early stages of growth, provided that it is supplied in a readily available form. After the plant is "topped" the absorption and utilization of nitrogen appear to be more harmful than beneficial. A marked absorption of nitrogen at this stage lowers the resistance and increases the susceptibility of the leaf to invasion by certain disease organisms. This condition is not so apparent if the rainfall is at a minimum during this period. Heavy rainfall, however, will greatly aggravate this condition.

Nitrogen is absorbed more readily than is potash under normal conditions. Moreover, excessive quantities of available nitrogen tend to depress the absorption of potash, a point which should be considered where quality production is involved.

Contrary to popular belief, Pennsylvania cigarleaf tobacco is not a high nitrogen tobacco. A concentration of 3.5% in the mature leaf will suffice if the concentration of potash is in excess of this figure.

Stable forms of nitrogen such as proteins contribute to certain qualities within the leaf itself. These compounds are not broken down during fermentation, and a relatively high concentration of proteins is not necessarily conducive to a good smoke. Simpler nitrogen compounds must be broken down and dissipated before the product is placed on the market. A high concentration of these may even prevent a satisfactory fermentation.

A good field of cigarleaf tobacco in Lancaster County, Pennsylvania

Potash irons out the leaf, giving it a smooth surface and improved texture. Left was fertilized with 1,000 lb. of complete fertilizer containing 16% potash; center with 1,000 lb. carrying 4% potash; right, no fertilizer.

It is quite apparent, therefore, that considerable attention should be given to the kind and quantity of nitrogen to apply. If a grower chooses to use 30 lbs. of nitrogen per acre, it is thought at the present time that 20 lbs. should be in a readily available form such as nitrate. On the other hand, 10 lbs. of the nitrogen should be in the form of cottonseed meal or its equivalent. This is to guard against adverse weather conditions, but excessive quantities of cottonseed meal must be avoided as the nitrogen is not in a readily available form and the chances appear good that more nitrogen will be made available through decomposition in the later rather than in the earlier stages of growth, which may be more harmful than beneficial.

Owing to the fact that these soils have been under cultivation for so long a period of time, it is quite possible and very probable that the concentration of certain elements necessary for the growth of tobacco has been reduced to a minimum. Among these may be mentioned manganese, boron, zinc, and a number of others of the less common elements. It is apparent, therefore, that a maximum development of a root system is necessary to insure an extensive feeding area which will tend to insure the absorption of the necessary quantities of these elements. This holds true likewise for potash, as the total absorption of this material appears to be closely associated with the extent of root development.

The grower has realized that a good physical condition of his soil is necessary for cultivation, aeration, and root development. In order to accomplish this purpose, and to obtain the maximum benefits of a fertilizer application, some growers have resorted to the use of well-rotted manure. This practice is to be highly recommended as here is found a quantity of elements, including nitrogen, in readily available forms. At the same time there is the further possibility that certain organic compounds may have a direct beneficial effect on the growth of the plants.

Other growers have resorted to applications of fresh manure to accomplish the same purpose. This practice is not to be recommended.

While fresh manure contains some nitrogen in a rather available form, a far larger quantity is present in unavailable form. Manure must undergo decomposition before the greater quantity of nitrogen is made available. As in the case of cottonseed meal, there is the possibility that more nitrogen is made available in the later rather than in the earlier stages of growth. Whether or not this deleterious effect may be avoided and the necessary physical effects obtained through the practice of adding the fresh manure to the corn crop preceding tobacco remain to be seen. If fresh manure must be used, it should be supplemented with a fertilizer mixture carrying a higher concentration of potash than is ordinarily recommended.

Other growers have sought to improve the physical condition of their soils by following clover or alfalfa with tobacco. Many years of experience lead one to believe that this practice is to be avoided, as these residues contain nitrogen in difficultly available forms. Evidence shows that more nitrogen is absorbed from legume residues in the later stages of growth. Moreover, some results have been obtained which showed more than 5% of nitrogen in the mature leaf, without any further nitrogen addition. Furthermore, the potash content of these leaves is usually at a very low level.

From what has been said it is evident that a considerable amount of attention should be given to the quantity and kind of nitrogen fertilizers to use for cigarleaf tobacco. It is apparent that organic carriers of nitrogen should be reduced to a minimum.

Many soils in Lancaster County are decidedly low in available potash, as indicated by characteristic symptoms of potash deficiency during the growing season. In these cases a marked increase in yield may be noted when this particular fertilizer is applied. Of far greater importance, however, is the effect of potash on quality.

When a limited quantity of available potash is present, this tends to become localized in the upper portions of the tobacco plant. If the proper quantity is available, there tends to be a more uniform distribution of this material throughout the plant.

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No element plays a greater role in the burning quality of tobacco than does potassium. Moreover, this element, in combination with malic and citric acids, appears to furnish a considerable amount of the energy necessary for the bacteria normal to the fermentation process.

The maximum benefits of a potash application are governed to a considerable extent by a number of environmental factors. The soil should be slightly acid, should contain a minimum of nitrogen organically combined, and should be properly balanced with respect to the other necessary fertilizer ingredients.

It is evident that the fertilizer treatment for a given soil should vary to some extent in a given locality. Excellent results have been noted throughout the County where a 4-8-12, 3-8-12, 3-12-12, 3-8-16 or 3-5-12 mixture has been used. It is quite evident, however, that it is not economical for a fertilizer manufacturer to place on the market a wide variety of tobacco fertilizers. At the present time, it is believed that either a 4-8-12 or a 3-8-12 mixture will meet the average conditions prevailing in this locality, on soil following corn, to which well-rotted manure has been applied in ample quantities and the fertilizer applied at the rate of 1,000 lbs. per acre. The cost of such an application is well within the reach of the average grower from a financial standpoint.

If these recommendations are followed out, other things being equal, a maximum concentration of potash is obtained in the leaves and a somewhat lower nitrogen content will prevail, a condition which favors quality and yield. At the same time the plants are far less susceptible to certain bacterial diseases which are common to the tobacco crop.

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STUDIES ON THE COMPOSITION OF BOVINE BLOOD AS INFLUENCED BY GESTATION, LACTATION, AND AGE[1]

WADARAN L. KENNEDY, ARTHUR K. ANDERSON,
S. I. BECHDEL, AND J. F. SHIGLEY

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In 1930 Anderson, Gayley, and Pratt (1), of this laboratory, published a paper on the chemical composition of bovine blood. In their work blood samples were taken at random from the dairy herd, the object being to determine normal values for many of the blood constituents. Perhaps the most interesting point brought out in their study was the wide variation in the glucose content of the blood of animals in the different age groups.

The work reported in this paper is a more detailed study of the effect of gestation and lactation on a few of the more important constituents of the blood which might be expected to vary under these conditions. There is also reported a detailed study of blood glucose values in calves' blood in an attempt to explain the high sugar values found as compared with the blood of older animals.

METHODS

Blood samples were taken between 6:00 and 7:00 A.M., usually before feeding. Two samples were drawn from the jugular vein, one in 20 mg. of potassium oxalate per 10 cc. of blood and the other without oxalate. The oxalated sample was used for the determination of non-protein nitrogen and glucose. From the sample without oxalate the serum was separated by means of a centrifuge within one hour after drawing and the serum used for the determination of calcium and phosphorus.

Non-protein nitrogen was determined by the method of Folin and Wu (7), and glucose by the method of Folin (6), on a laked blood filtrate. In most cases the glucose method was modified by using a standard containing 0.1 mg. of glucose per 2 cc. This was necessary because the glucose content of cow's blood is about one-half that of human blood.

Serum was analyzed for calcium by the method of Clark and Collip (4), and for phosphorus by the method of Youngburg (24).

In the studies on carbohydrate tolerance the method of Killian (13) was

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[1] Paper No. 847 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This paper is part of a thesis presented by Wadaran L. Kennedy to the faculty of the Graduate School of The Pennsylvania State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

used. A technical grade of glucose was dissolved in water to make a 50 per cent solution and administered as a drench.

EXPERIMENTAL

1. *Influence of Gestation and Lactation on the Composition of Bovine Blood*

Since the complete analysis of blood requires much time, it was felt that the work might be lightened by a careful selection of determinations to be made. Many of the determinations made in a blood analysis are for nitrogenous constituents which are included in the non-protein nitrogen fraction. Therefore it was felt that non-protein nitrogen values would indicate whether or not any of the components of this fraction were varying. A knowledge of non-protein nitrogen values would also serve as an excellent measure of kidney function throughout the experimental period. Since previous work (1) had indicated that the glucose content of bovine blood varied considerably with age, it was felt that this determination should be included. Calcium and phosphorus determinations were included because during pregnancy and lactation extraordinary demands are placed on the reserves of these elements in the body.

In this study the blood of the experimental animals was analyzed at approximately weekly intervals from the time of breeding through one year of lactation. Since the time required to follow the same group of animals throughout the entire period of gestation and lactation was prohibitive, two groups of animals were used. Group I consisted of five heifers (two Holsteins, two Guernseys, and one Jersey) that had been bred for the first time and Group II consisted of five cows (four Holsteins and one Jersey) that had just dropped their first calf. The animals were selected from the purebred dairy herd of The Pennsylvania State College. They received the regular ration fed the dairy herd. All samples were analyzed in duplicate and the average of these results was used.

The data obtained in this study indicated that gestation and lactation had little effect on the blood constituents determined. Since the presentation of these data would require a table containing over 1,300 values, it appeared desirable to summarize the data in Table 1 and comment on a few of the results that perhaps are significant.

Non-protein nitrogen.—Non-protein nitrogen values ranged from 21.01 to 45.71 mg. per 100 cc. of blood, the average value being 32.82. These values do not differ significantly from those which one would expect to find for normal human blood. The average value during gestation is about 2.0 mg. less than the average value during lactation. The results agree with those of Anderson, Gayley and Pratt (1) and Hayden and Sholl (10).

TABLE 1

Showing the composition of cow's blood from the time of breeding through one year of lactation

Number of animals	Constituents determined	Number of determinations	High	Low	Average
From breeding to parturition					
				mg. per 100 cc.	
5	Non-protein nitrogen	164	45.71	21.01	31.83
	Glucose	171	68.00	60.70	52.51
	Calcium	166	12.90	7.70	10.73
	Phosphorus	161	7.54	4.16	5.64
From parturition through lactation					
5	Non-protein nitrogen	168	45.58	21.24	33.82
	Glucose	171	52.33	37.80	51.73
	Calcium	166	13.20	9.00	11.08
	Phosphorus	164	7.49	3.38	5.23
From breeding through lactation					
10	Non-protein nitrogen	332	45.71	21.01	32.82
	Glucose	342	68.00	37.80	52.12
	Calcium	332	13.20	7.70	10.91
	Phosphorus	325	7.54	3.38	5.44

Immediately after parturition non-protein nitrogen values were above average, the values of the five animals being 34.28, 39.09, 41.73, 43.33 and 44.11 mg.

During a period of severe cold weather, where the temperature remained below zero most of the time for about three weeks, non-protein nitrogen values were from 5 to 10 mg. higher than they were just previous and just after this period. Since the animals did not drink as much water as usual during the cold weather, this may explain the high results. Peters and Van Slyke (16) state that non-protein nitrogen values in human blood are increased with a low water intake.

Glucose.—The results for glucose gave an average of 52.12 mg. per 100 cc. of blood for 342 determinations. Values ranged from 31.8 to 71.4 mg. The low value is questionable, since for the previous week this animal gave a value of 46.6 mg. and on the following week 50.7 mg. At no other time throughout the experiment did the value for this animal go below 46.2 mg. The second lowest value for any animal was 37.8 mg. The average value of 52.12 mg. is in close agreement with that reported by Hodgson, Riddell and Hughes (12); Anderson, Gayley and Pratt (1); and Hayden and Sholl (10).

There was no significant difference in the glucose content of the blood of the pregnant non-lactating animals and of the lactating group. The former group gave an average of 52.51 mg. while the latter gave 51.73 mg. Considerable work has been reported on the effect of lactation on blood sugar values. Among those who have found low values for lactating animals may be mentioned Widmark and Carlens (23); Hewitt (11); Hodgson, Riddell and Hughes (12); Schlotthauer (19); Schwarz (20); Schwarz and Mezler-Andelberg (21). Moussu and Moussu (15) and Fish (5) report higher values for lactating cows than for dry cows. Scheunert and Pelchrzim (18); Awdejewa, Prowatorowa, Sawitsch, and Thal (3); and Hayden and Fish (9) found no difference in the blood sugar level between dry and lactating cows.

Calcium.—The values for calcium on blood serum were fairly constant throughout the experiment, most of the values falling between 9.0 and 11.5 mg. per 100 cc. As a result of 332 determinations, values were obtained ranging from 7.7 to 13.2 mg., the average value being 10.91 mg. per 100 cc. of serum. Pregnancy and lactation apparently had little effect on the calcium content of the blood, the average value for the former period being 10.73 mg. and for the latter 11.08 mg. Our results agree with the findings of Meigs, Blatherwick and Cary (14); Robinson and Huffman (17); and Haag (8).

Phosphorus.—The values for inorganic phosphorus in the serum remained fairly constant throughout pregnancy and lactation. As a result of 325 analyses, values were obtained ranging from 3.38 to 7.54 mg. per 100 cc. The average value was 5.44 mg. per 100 cc. During pregnancy the average value was 5.64 mg., while during lactation it was 5.23 mg. In previous work from this laboratory (1) it has been shown that serum phosphorus values decrease with age. Since in this experiment we were dealing with young animals bearing their first calf, it is quite possible that the slightly lower values for phosphorus during lactation are due to an age factor rather than to lactation. The above values agree closely with the results obtained by others (1, 14, 17).

2. *Blood Sugar Studies*

If one compares the composition of human blood with that of the cow, the most striking difference is found in the sugar content. Normal human blood contains in the neighborhood of 100 mg. of glucose per 100 cc. whereas cow's blood contains approximately 50 mg. per 100 cc. When the glucose value of human blood reaches 45 mg. per 100 cc. it is said to be at the convulsive level. Why a cow can be normal with the blood sugar at the convulsive level for humans is an interesting question. Since sheep (22) and goats (9) also have low blood sugar values it would appear that a low level of blood sugar is characteristic of ruminants. Dogs and horses (9) have blood sugar values approximating that of man, while chickens (9) and rats (2) have definitely higher values than man. From studies with the use of insulin in diabetes it is known that there is a definite relationship between the amount of insulin given to an individual and his blood sugar level. It would be an interesting study to determine the insulin content of the pancreases of different species and to observe any correlation between these findings and the normal blood sugar levels for these species.

Blood sugar and age.—A very interesting fact brought out in a previous study (1) of the blood sugar of bovines was that in young calves blood sugar values are twice as high as in mature cows. This has suggested a more detailed study of the influence of age on blood sugar values. For this study two groups of calves were selected; the first group consisting of three calves less than a week old and the second group of two calves five months of age. The two groups were selected in order to cover approximately the first year of life in the six months available for the work. Samples were taken usually at 8:30 A.M. after feeding and at weekly intervals covering the first 47 weeks of life. The results are shown graphically in Figure 1.

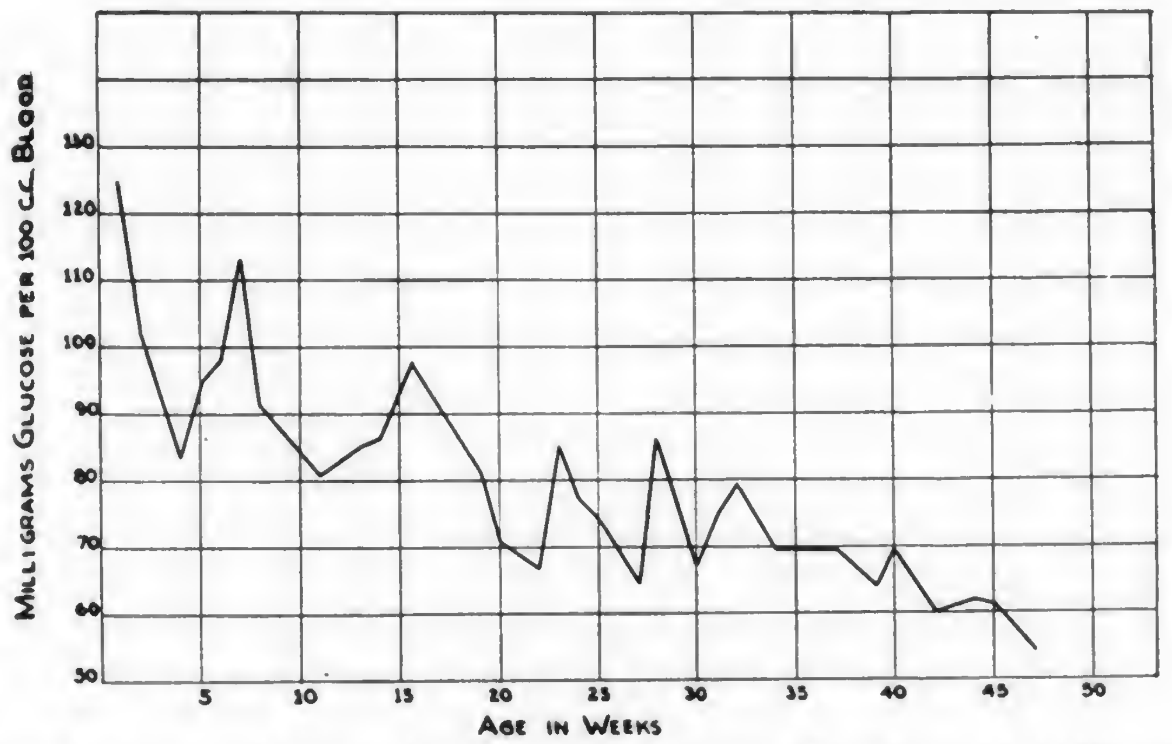

FIG. 1. Showing variation in blood sugar values of calves as influenced by age.

It may be observed from Figure 1 that for the first week of life the average blood sugar value was 125.0 mg. per 100 cc. of blood. By the end of the second week the average value had dropped to 102.5 mg. and by the end of the fourth week to 83.3 mg. For the next three weeks values increased and then they decreased. Considerable variation was observed until the thirtieth week when the curve became quite smooth to the end of the period when a value of 54.4 mg. was reached, which is about the value to be expected for a mature animal.

Diurnal variation in blood sugar.—The fluctuations in the first half of the curve shown in Figure 1 suggested a possible diurnal variation in blood sugar values. In order to study this an experiment was run on two calves over a period of 24 hours. Blood samples were taken frequently throughout this period. The results are shown graphically in Figure 2. The first samples were taken before feeding in the morning and the last samples before feeding the following morning. The blood sugar averaged 80.8 mg. per 100 cc. on the first morning and 80.5 mg. on the second. Samples taken one hour after the morning feeding showed an average glucose value of

129.4 mg. per 100 cc. This value gradually decreased until at 2:00 P.M. the value was 86.6 mg. The calves were fed again at 4:30 P.M. and blood samples were drawn again at 5:00 P.M. At this time the average glucose value was 114.2 mg. The reason that this value did not equal the level of 129.4 mg., reached one hour after feeding in the morning, is undoubtedly due to the fact that only 30 minutes elapsed from the time of feeding until the blood sample was drawn in this case. By 11:30 P.M. the glucose value had decreased to 96 mg.

From this experiment it is readily seen that there is a marked diurnal variation in the blood sugar values of calves which is associated with feeding. Since the calves were being fed mainly milk, the high blood sugar values may easily be accounted for by the high lactose content of the diet.

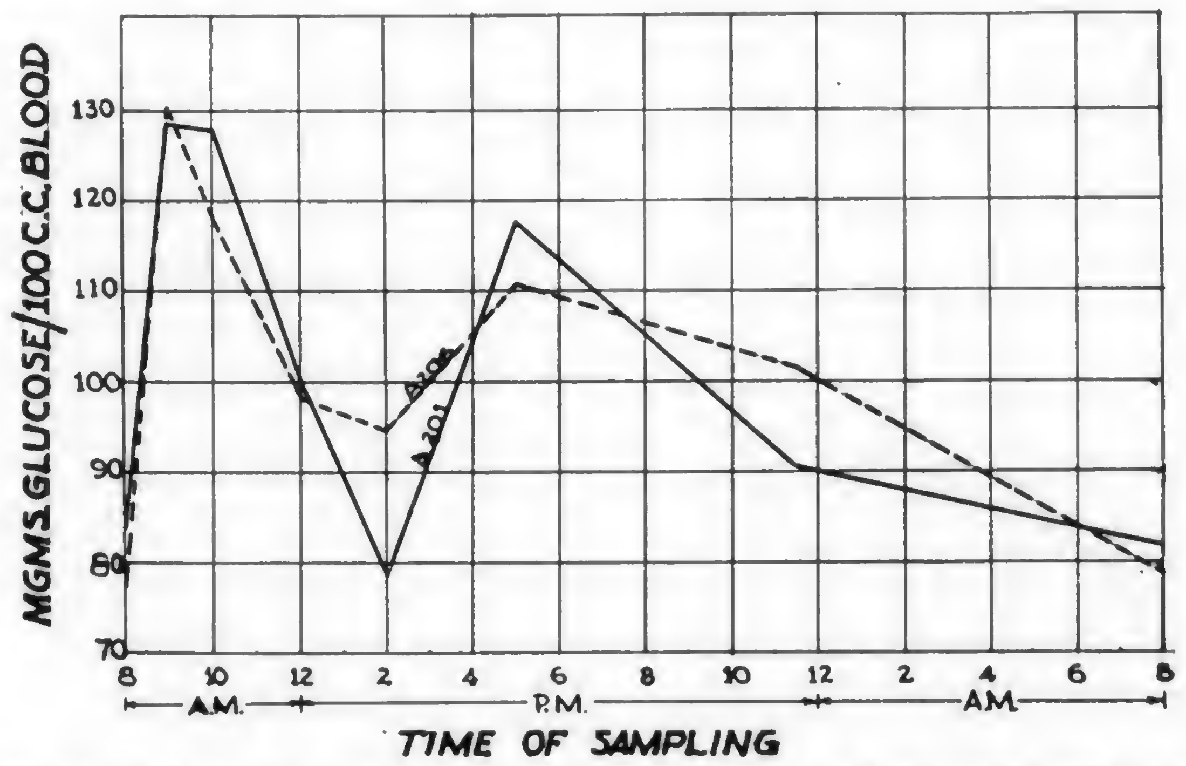

FIG. 2. Showing diurnal variation in blood sugar of calves less than one month of age.

Each calf received 13 pounds of milk a day which would contain about 0.6 pound of lactose. Thus at each feeding a calf received about 0.3 pound or 135 gm. of lactose. If in the case of a calf, milk passes directly into the abomasum and shortly into the intestine, there would be little delay in the digestion and absorption of lactose after milk is consumed.

When a carbohydrate tolerance test is given in human medicine the patient is given 1.75 gms. of glucose per kilogram of body weight. Since the average weight of the calves in this experiment was 87 pounds, the amount of sugar each received per feeding amounted to about 3.4 gms. per kilogram of body weight or about twice the amount given to a human in a sugar tolerance test. This would easily account for the rise in blood sugar after feeding. It is evident from this experiment that blood sugar values in calves must be interpreted on the basis of time of sampling in relation to time of feeding.

Sugar tolerance tests.—Although the feeding of large quantities of milk accounts in large measure for the high blood sugar values observed in the above experiment, it does not account for the fact that even before feeding, calves have higher blood sugar values than mature cows.

In human nutrition it is recognized that the digestive mechanism is not highly developed in an infant. An infant may thrive on its mother's milk but may fail to develop properly on a modified cow's milk. This may be due to the fact that the infant has the digestive enzymes capable of handling human milk while it may be lacking in enzymes capable of digesting the slightly modified molecules in cow's milk. It is quite possible that the same situation holds with regard to the endocrine system of a young animal. If the pancreas of a young animal produces a limited supply of insulin, this might easily account for the high blood sugar in young calves. It is possible that a calf may have diabetic tendencies.

In human medicine, when a person is suspected of having diabetes, it is customary to make a carbohydrate tolerance test in which 1.75 gms. of glucose per kilogram of body weight is fed to the individual and the influence of such treatment on the blood sugar level is determined by frequent blood analyses. In order to determine whether or not calves have less ability to utilize sugar than cows, the following carbohydrate tolerance tests were made. The results are shown graphically in Figure 3.

Eighteen animals ranging in age from less than one month to mature cows were divided into eight groups on the basis of age as indicated in Figure 3. Each animal was given a carbohydrate tolerance test by feeding 1.75 gms. of glucose per kilogram of body weight administered as a drench in a 50 per cent solution.

Immediately before feeding the sugar meal blood samples were taken and analyzed for glucose. After the sugar meal, blood samples were drawn at the end of one, two, three and four hours and likewise analyzed for glucose. The values are plotted in Figure 3 for each animal.

It is evident from Figure 3 that animals less than one month of age show a much less rapid utilization of glucose than the animals of the older groups. As age increases there is better and better utilization of glucose until in the bred heifer and lactating cow groups there is only a slight elevation of blood sugar values after the sugar meal. Animals A–202 and A–187 do not appear to fit into the general picture. In the case of A–187 a possible explanation may be that this calf had been allowed to nurse different cows until it was four months of age and thereby may have developed the ability of taking care of more sugar than the other calves. It should also be noted that both A–202 and A–187 started the carbohydrate tolerance test at much lower blood sugar levels than their mates. It is possible that this low initial blood sugar level indicates a glycogen depletion at the outset of the test and that the sugar fed was used immediately for the production of glycogen. However the odd responses of these two animals may be more

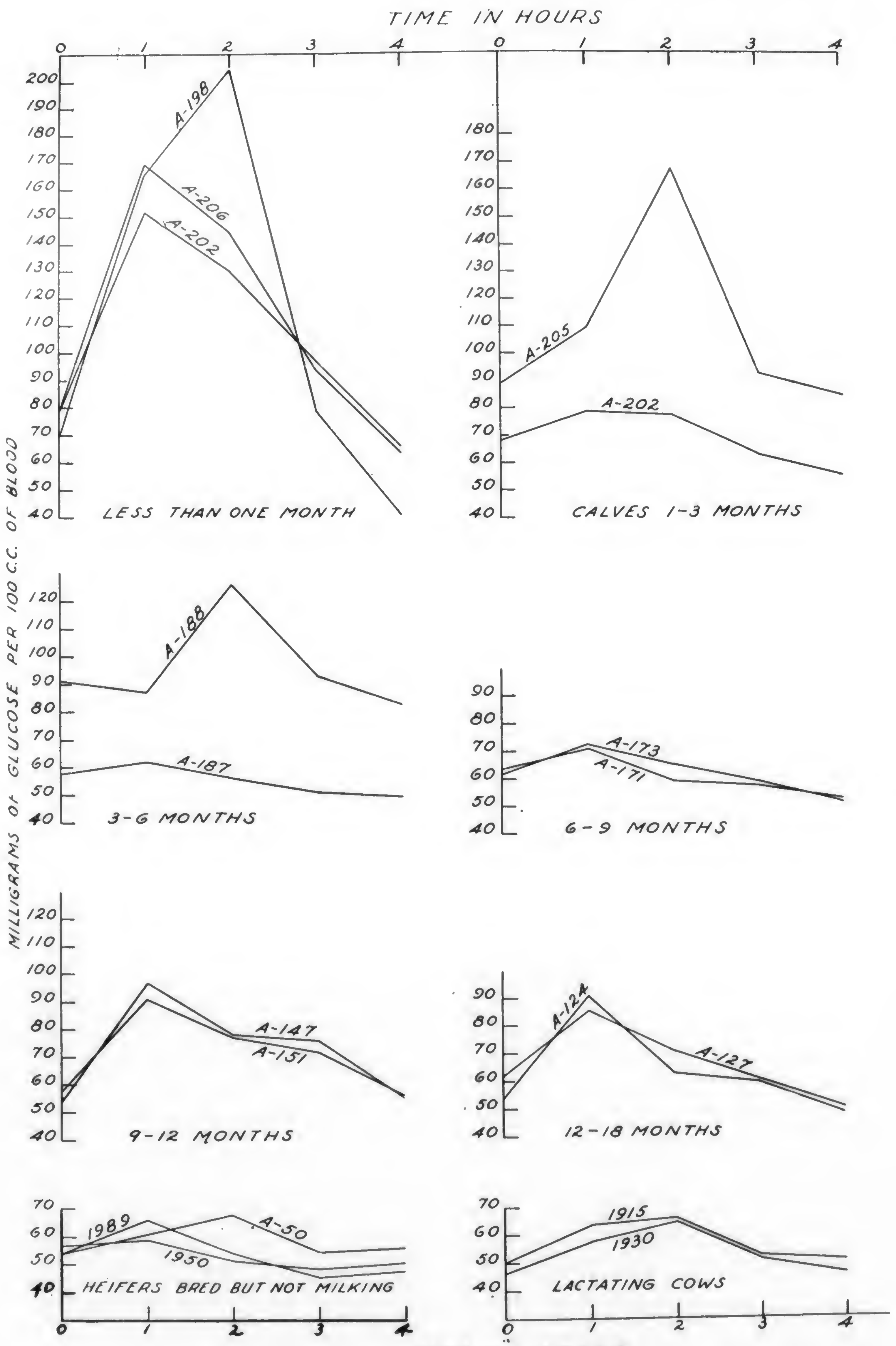

FIG. 3. Showing results of sugar tolerance tests on animals of various ages.

simply explained by assuming that they have developed an efficient glucose utilization mechanism at an early age.

From this experiment it is apparent that calves, especially very young calves, are less able to metabolize glucose than older animals. It would appear that the most logical explanation is that in very young calves the pancreas is not producing as much insulin as in older animals. It would be an interesting study to compare the insulin content of the pancreases of young calves and older cows. If it were found that the pancreases of older animals were richer in insulin than those of young animals this information might find a valuable application in the commercial manufacture of insulin.

SUMMARY

The blood of heifers has been analyzed at weekly intervals, from the time of breeding through one year of lactation, for non-protein nitrogen, glucose, calcium and phosphorus. There is little variation in these blood constituents as influenced by gestation and lactation. Average values for non-protein nitrogen were 32.82; for glucose 52.12; for calcium 10.91 and for phosphorus 5.44 mg. per 100 cc.

Blood glucose values are definitely related to age. Blood from calves less than one week of age contained 125 mg. of glucose per 100 cc. This value gradually decreased to 54.4 mg. per 100 cc. of blood at the end of a year.

There is a diurnal variation in the glucose content of calves' blood. Directly after feeding milk, blood sugar values increase sharply.

Carbohydrate tolerance tests on animals of various age groups indicate very little response to sugar feeding in the older groups but a decided response in young calves.

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OUR DEB

Editor's Note: This articl
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OUR DEBT TO EVAN PUGH

by R. Adams Dutcher

Editor's Note: This article is the first in a series by the author in which he deals with the life of one who had much to do with laying the foundation on which the educational and research program of Penn State was built. Dr. Dutcher first presented the material herein contained at an initiation banquet of Gamma Sigma Delta. He has put it into print at the special request of Dean R. L. Watts. The author wishes to acknowledge the use of the research papers published by Dr. Runkle, the College Historian, on the life of Evan Pugh.

WE are all proud of the reputation Penn State has achieved in teaching and research but I feel that few of us appreciate the work, worry and sacrifice of those who have gone before and who have made Penn State what she is today. I have chosen to write of a man who, more than any other single individual, is responsible for the policies and ideals which we have come to know as characteristic of our institution. This man is Dr. Evan Pugh, first president of the Pennsylvania State College and one of the most distinguished agricultural chemists and educators of his time.

Like the brilliant French scientist Lavoisier, Pugh died in his thirties and, like Lavoisier, the shadow of his genius will persist through the years.

Born in February, 1828, in Chester County, Pennsylvania, he attended district school for a time but most of his early education was obtained by home study and private tutelage. His father, who was of Welch-Quaker descent, died in 1840, leaving a small farm and a blacksmith and wheelwright shop. His grandfather, who was made his guardian, apprenticed him to a blacksmith with the hope that he would follow in his father's footsteps. For two unhappy years (until he was 18) he served his apprenticeship.

He finally purchased his release and attended the "Manual Labor Academy" at Whitestown, New York, where he studied Analytical Geometry, Differential and Integral Calculus, Astronomy, Botany, Physics, Geology and Chemistry. He returned from the Academy with the definite conviction that teaching should be his life work. Consequently, he transformed the second story of his father's shop into a school which he called the Jordan Bank Academy.

During this period he was unconsciously forming ideas that were to influence his actions and thinking for the rest of his life. While he taught his students, he was busy managing the estate, directing the planting and harvesting of crops and cogitating on the applications of science to agriculture and successful farming. In 1853, as a result of good management and industry, he disposed of the property for $2800 and prepared to embark for Germany, France and England with the view of pursuing his studies and investigations. While all available records point to the fact that he read everything he could find on scientific agriculture, there is no evidence that he had attempted any type of investigational work—except those practical experiments which related to crop production.

Dr. Evan Pugh, first President of the Pennsylvania State College.

He not only read in the fields of science and agriculture but he was particularly interested in history, tales of the sea and certain types of novels popular in that day. He also became proficient in shorthand. His letters, papers and lecture notes are interspersed with shorthand notes which he used to good advantage in German universities.

At 25 years of age he had, largely by self education, learned to express himself with ease and clarity. He was a good speaker as a result of his teaching experience and his written prose and verse attest to his versatility and natural ability as a writer.

On September 7, 1853, he sailed on the George Canning for Hamburg, Germany. The voyage lasted nearly a month and the following letter relating to the voyage is characteristic of the man. He is approaching the English Channel.

"Last night I consider our most hazardous night (over three weeks on the sailing vessel George Canning) and it speaks much for the science of navigation that we could cross an ocean 3000 miles wide and after being blown in almost every direction by contrary winds and drifted by oceanic currents and overshadowed by dense fogs for days, that by getting a faint glimpse of the sun yesterday afternoon at two o'clock when it was scarcely visible through the clouds we were ready to enter a narrow channel at the only safe place of entrance for hundreds of miles and then to proceed up it as we have done thus far without seeing land since we lost sight of Long Island in the United States.

"This is what reason, human reason can do, and yet men say, 'It's very dangerous to exercise it.' It will lead to infidelity. O, shameful detraction of God's noblest, richest, choicest gift to man. If there is one element of His nature in which God is personified more than another, if there is one characteristic that speaks more plainly than another, that man is created in the image of his God, it is! it is! that of Reason, 'Human Reason,' God created, Heaven Gifted Human Reason."

He spent a year and a half at Leipzig studying Geology, Minerology, Microscopy and Botany. It was here that a delightful friendship sprang up between Pugh and a fellow American student by the name of Samuel W. Johnson. Both young men had similar aims and ambitions, viz., to return to America where they could put into practice the new system of education which dignified labor without

(Continued on page 186)

ALUMNI NOTES

by Kenneth F. Moist, '42

Agronomy

Clarence Henry '09—is educational director for the Chicago Board of Trade. He recently delivered a number of lectures at the Pennsylvania State College.

R. I. Throckmorton '11—for many years has been head of the Agronomy Department of Kansas State College of Agriculture.

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R. D. Lewis '19—received his Ph. D. degree from Cornell in 1926 and is now in Agronomy Extension work at Ohio State.

R. H. Engle '21—who had been for some years with the National Lime Association, now has a position with the National Fertilizer Association.

Jesse D. Romaine '23—has been for some years Chief Agronomist in the American Potash Institute. His business address is Investment Building, Washington, D. C.

J. D. Ryburn '29—is teaching vocational agriculture at Picture Rocks.

John J. Noll '34—has been in flood control division of the U. S. Soil Conservation Service. He is now located at Connellsville.

Leroy Miller '36—is now a graduate student at Michigan State College and expects to get his M. S. degree in June.

Claude E. Custer '38—is Camp Agronomist at a CCC Camp at Shelocta.

John O. Rigo '38—is Junior Agronomist in the U. S. Soil Conservation Service. His address is White Hall, Md.

A. Rudolph York '38—who was graduated in mid-year is Supervisor in the Farm Security Administration, for Elk, Jefferson, Clearfield, Cameron and McKean Counties. He is located in Du Bois.

Hugh C. Watts, '38—is a teacher of vocational agriculture at Souderton, Montgomery county.

Poultry Husbandry

Henry Body '23—is operating a large poultry establishment at Yellow House.

C. S. Platt '23—is Professor of Poultry Husbandry, New Jersey State College, New Brunswick, N. J.

J. A. Davidson '24—intercollegiate wrestling champion in the 175 pound class. He is now in charge of poultry research work at the Michigan State College, East Lansing, Mich. Davy is working toward his Doctor's Degree.

Benj. L. Sheaffer '24—Lancaster, R. D. 3., is one of the best salesmen in the state of Pennsylvania.

F. P. Jeffrey '32—is doing a fine job of teaching at the New Jersey State Agricultural College in the Poultry Department at New Brunswick, N. J.

Warren D. Johnson '34—Nottingham, will some day be the White Holland turkey king of Penna. He has a splendid flock of turkeys and captured many prizes at the recent Farm Products Show in Harrisburg.

P. F. Rubinkam '34—Sellersville, is showing the boys the value of a college education through the establishment of a first class poultry breeding farm.

Chas. Northup '36—is now employed by the Borden Company in sales work with particular reference to poultry feeding. He has a territory embracing six states.

C. D. Musser '37—is now employed by the Purina Mills of St. Louis, Mo. Watch his smoke as he starts to sell you Purina feeds.

A. C. Groschke '38—is pursuing graduate study at the University of Maryland, College Park, Md.

Ag Engineering

James V. Baker, '35—secured employment in the buying office of Sears, Roebuck and Company at which place he worked until last fall when he was promoted to the Technical Department of the firm. He is located in Philadelphia.

Waldo E. Bell '34—is working on soil conservation. He is doing a fine job at Waynesboro where he is employed by the Soil Conservation Service.

E. K. Bonner '35—is in the employment of the A. B. Farquhar Company operating in York.

Frank R. Brower '37—got stung by Cupid's dart and on April 16, 1938 ended his career as a single man. The International Harvester Company was well pleased with his work for he recently was changed from sales promoter to a better job; however, he is still doing some work as a sales promoter along with his employment in the service department of that company. He is situated at Elmira, New York.

Harvey F. Corson '36—has found work as a blockman for the Allis-Chalmers Company located at Brookville.

E. Robert Curry '36—is working for the Philadelphia Electric Company at West Chester.

George W. Grisdale Jr., '35—of Freehold, New York, is the Project Hydraulic Engineer for the Soil Conservation Service of that town. He is in charge of Research and Hydraulic Studies on the Freehold Project. Those who know him will be pleased to learn that he is now married and has a family.

E. E. Houck '37—is one of the engineering Staff of the Oliver Farm Equipment Company of South Bend, Indiana.

John P. Lonberger '36—is doing sales work for Goulds Pumps Inc. of Pittsburgh.

Richard T. Markle '36—is working for his father on the home farm near State College.

G. E. Oesterling '33—is another fortunate Ag. Engineer. He is doing fine work for the Soil Conservation Service in a C. C. C. camp in the Soil Conservation District near Glenn Rock.

Luther Singley, '37—has gotten employment with the West Penn Power Company of Central Pennsylvania.

Murrel E. Strickler '34—has a good job as a draftsman in the drafting room of the John Deere Harvester Works of East Moline, Illinois.

William C. Wenner '35—is employed by the Pennsylvania Power and Light Company located at Stillwater.

Paul A. Whisler '36—has gotten employment with the Allis-Chalmers Company of LaPorte, Indiana.

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out the season and analyzed in order to get a measure of the total grass and the total digestible nutrients produced during the season.

Plant population estimates of the important species are made both spring and fall in order to study the botanical changes brought about by various fertilization and management practices in use. The following table shows some of the changes in plant population since the establishment of the pastures:

A study of the accompanying table showing the returns per acre above feed and fertilizer costs and the cost of the time required to apply the fertilizers will readily convince even the most skeptical that every penny spent for fertilizer applied to the pastures brought a very profitable return. For the five years shown in the table an investment averaging $1.92 per year for the superphosphate applied returned an average of $18.04 annually.

None of the other treatments returned as much for the amount invested in fertilizer but it can easily be seen that any of the treated pastures gave a very good return on the invested money in addition to the fact that all of them produced at least twice as much milk as the one receiving lime only, and the quality of the herbage was much better.

The matter of deciding just which fertilizer treatment to apply for most economical results should be considered rather carefully. If there is a large acreage of permanent pasture on the farm which might produce more grass than could be utilized under a program of heavy fertilization, it may be advisable to confine the fertilizer application to phosphate or phosphate and potash.

On the other hand, if there is a relatively small area of pasture and very high production is desirable, it may be possible to get three or four times as much grass as usual by heavy applications of a complete fertilizer or the use of plenty of minerals along with barnyard manure.

Undoubtedly pasture fertilization has been proven profitable from any standpoint. The application of knowledge gained through this research project should be of great value to every livestock farmer and dairyman.

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In cooperation with the Block and Bridle Club, the "Farmer" will devote the May issue to Animal Husbandry

Our Debt To Pugh

(Continued from page 167)

losing sight of the cultural. A scientific correspondence ensued which was only terminted by the death of Dr. Pugh.

To give you some idea of the factors that were influencing the lives of Johnson and Pugh, permit me to quote from a letter of Johnson written in 1855 regarding his impressions of the Academy of Agriculture and Forest Culture at Hohenheim, Germany. At this time such institutions were almost unknown in America.

He states, "The pupils are mostly from wealthy families. A finer set of young men I have not seen elsewhere in Europe. They are described as full of zeal in the prosecution of their studies, and when contemplating their animated and intelligent countenances, I could but hope that the day will shortly come when the United States shall also engage her intelligent sons in similar preparation for similar duties. Most of the graduates of this Academy are afterwards occupied with the management of great estates . . . .My stay in Hohenheim was rendered peculiarly agreeable by the kind attentions of Prof. Wolff, whose acquaintance I had made in Moeckern, near Leipsic. Hohenheim has never had until now a man among its faculty who has united eminent scientific ability with practical knowledge. It has been fortunate for the Academy that practice has hitherto held there such dominant sway. It is equally fortunate for it that it now has a teacher who is able to show that practice and science harmonize in most points, and who labors not so much to immortalize himself by propounding and defending ingenious theories, as to advance practical agriculture by attempting the solution of the problems that are encountered in daily life.

Prof. Wolff has had fitted up a spacious though simple Laboratory in which he instructs a goodly number of the pupils in chemical analysis. He finds that when the course is rightly conducted, it proves highly interesting, as it cannot fail to be highly profitable for the pupils. It is in the Laboratory, and from the teachings of the capable agricultural chemist that agriculture is to make rapid advances in its doctrines and literature, while from practical instruction, the existing good systems are to be brought into general application. Hohenheim has hitherto been efficient in diffusing agricultural knowledge; hereafter it cannot fail to become as eminent for discovering new truths, which shall make itself progressive as it now makes Wurttemberg and the surrounding countries so . . . .

Hohenheim is doubtless the best school of agriculture that exists, and the agriculturists of the United States, who are founding agricultural colleges, etc., will do well to send out a few young men to take the course here, as part preparation for stations as teachers at home."

It is evident that Pugh and Johnson had been discussing the organization of an American agricultural school because Pugh wrote Johnson in 1855 as follows:

"In reply to your questions with regard to American Agricultural School . . . .I sympathize to the fullest extent with the spirit of the enterprise you propose . . . .With respect to your distribution of the studies and the length of the term, you are in advance of my plans . . . .To sum up: I enter with my heart and hand into the spirit of your proposal excepting that I would rather delay at least one year (to Sept. 1857) . . Of the success of the institution I have no doubt if it once gets started on the basis you propose."

He then spent six months at Heidelberg where he studied with Bunsen and other well known chemists and mathematicians.

Pugh spent an additional year at Gottingen working with Wohler in organic chemistry and perfecting his mathematics. In one of his diaries Pugh shows that he was "on his toes" so far as familiarity with scientific literature is concerned. In searching for a thesis subject for the doctorate he suggests a number of problems but these are vetoed by Wohler on account of expense. Wohler suggested a quinoline problem which Pugh maintained had already been solved by Hoffman of London. Not until Pugh presented Wohler with the evidence, from an English chemistry text, would Wohler believe that Pugh was correct.

This was not the only instance in which the embryonic American chemist was able to prove that the famous Wohler was not infallible. Wurtz had just discovered his famous synthesis in which alkyl radicals could be joined by treating alkyl halides with sodium. Wohler suggested that similar reactions might work using iodine cyanide. Pugh pointed out that sodium would combine with both iodine and syanogen and—as Pugh put it—"the cake would be all dough again." They finally compromised on a mineralogical problem dealing with composition of minerals from Mexico and Central America. The thesis was entitled "Miscellaneous Chemical Analyses."

One of Pugh's German friends, formerly privatdocent at Leipzig, had gone to France to work with Ville on plant nutrition problems. Returning to Germany on a visit, he described experiments (to Pugh) in which Ville has "doctored" results in order to prove that nitrogen assimilation by plants was, at least in part, a question of utilization of gaseous nitrogen from the atmosphere. De Saussure had already advanced the idea that nitrogenous compounds are taken up almost entirely through the root systems of plants. Boussingault had also fought the same battle but with greater analytical precision as a result of better technics.

Pugh not only sided with Boussingault but his hatred of sham and dishonesty made him doubly anxious to do more research to settle the matter once and for all and, at the same time, "show up" Ville and discredit his work. Some observers might criticize Pugh for this vindictive attitude but his was the zeal of a crusader in science—Truth for truth's sake. The spirit was not unlike that of many early crusaders who felt it their duty to expose pagan philosophies. Consequently, on his trip to Paris he went out of his way to "check up" on all phases of the problem and he became convinced that he could plan a series of experiments that would definitely establish the correctness of his hypothesis.

As a result he addressed a letter to Dr. Gilbert, a former student of Justus von Liebig, who was Director of Research for Sir John Lawes at the Rothamsted Experiment Station in England. His suggestions were greeted with enthusiasm by Gilbert and arrangements were made for him to delay his return to America in order that he might conduct his experiments at Rothamsted. In a letter written to Johnson in August, 1857, he described with enthusiasm the facilities placed at his disposal. Twelve glass shades (each 3½ feet high by 9½ inches in diameter) were provided—in which plants were grown in NH_3 free air. He mentions the fact that many distinguished visitors came to the laboratory to see the experimental set up and he mentions with somewhat fiendish glee—"Ville will be here next week."

Considering the knowledge and technics available at that time,—the work was very well planned, the apparatus ingeniously devised and the entire experiment was conducted with great care and accuracy. There is little question that Pugh would have made a world wide reputation had he been permitted to devote his talents to research. As it was, the publication (in 1860) of the results of the investigation, showing that gaseous N_2 and NH_3 are not assimilated by plants, gave him a high rating as a research worker.

Lawes and Gilbert offered Pugh a permanent position at Rohamsted but he felt that two years at Rothamsted had given him the background he needed for further useful service in America. He had been elected a member of the British Chemical Society and his work had received world recognition when published by the Royal Philosophical Society of Great Britain.

He had dedicated his doctor's thesis to a prominent American plant scientist, Dr. William Darlington. Dr. Darlington wrote Pugh, in 1855, thanking him for the honor and opened the question of a Pennsylvania Agricultural School. He states, "As our State Agricultural Society has procured a farm for the instruction of young men in the science of Agriculculture,I should hope they will, ere long, make provision for teaching Agricultural Chemistry—and thus create a demand for the knowledge which you have gathered in Europe."

Not only had Johnson and Pugh discussed the possibilities of such a school but Johnson had actually been approached by Dr. A. L. Elwyn of Philadelphia, one of the original trustees of the Farmers High School. Johnson had recommended Pugh for the post, since he planned to return to Yale. Elwyn must have negotiated with Pugh as early as 1855. The following letter from Pugh to Johnson was written in 1854 before negotiations had actually been opened with Pugh.

He states, "I feel an enthusiastic interest in the success of this College, even though I should never be in the slightest degree connected with it. My plan was to labor for its establishment and if I had the honor of participating in the duties it would require of a teacher and Professor—good. If I did not receive the honor and it was conferred upon others who would make it what it ought to be—then also good. I would labor through the instrumentality of friends to build up an auxiliary establishment in connection with the College and thus go one step toward completing my ideal, a large general centering point for the State College with its auxiliaries in every county, with its investigation stations in every township and soon with its observers on every farm."

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EDITORIALS

Congratulations, AG COUNCIL!

The Agricultural Student Council surely deserves commendation for its recent achievements—the opening of the Ag library on Sundays and the representation of the Council at the Ag school faculty and executive committee meetings. We feel that this action is indicative of the closer understanding between the faculty and the student body; and this is a large forward stride toward a mutual cooperation between the instructors and the students, which is so necessary to successful present day education.

We further feel that this achievement is an answer—and a very good answer—to the challenge recently hurled at the extra-curricular activities to justify their existance. Here is definite evidence that the Ag Council, for one, has a reason for its being—to serve as a medium for the unification of the various clubs and organizations in the Ag School and as such, to promote better student-faculty relationships. Here is evidence that the Ag Council is more than a key collecting society; more than a mere social gathering. Here is something that will benefit the whole Ag School, and will serve as an example for the other Schools of the College.

Last Fall the Council held the annual Harvest Ball, and made it the greatest success ever seen in the Ag School. Now the Spring counterpart, the Ag Frolic, is under way. We hope the Council, with its few members and officers, will stick together as they did last fall and make the Frolic even more successful. It's up to you as a group, and to each member as an individual, to do his part. . . We wish you luck!

On the other hand, we still feel that the dinner-dance at Skytop did not serve the purpose for which it was avowedly intended, namely to acquaint the new Council members with each other and with the old members. We admit that it was a well attended and enjoyable affair—some stating that it was the best dance this year. However, few new members got to know their fellows and the old members of the Council. Almost everybody became well acquainted with the small group in which he found himself and with the girls in the group. Reliable reports indicate that the roll book and directory are still quite essential to connect names with persons. While we have no fault to find with the dinner-dance itself (in fact, we rather favor the idea), we can certainly not agree with the spirit in which it was held. We would far rather see an open declaration of having a good time than a flimsy excuse about getting acquainted, since this is virtually impossible at a semi-formal affair of this nature.

S. B. G.

TOP STAFF POSITIONS OPEN TO ALL

At a recent meeting the board of the Penn State Farmer voted to open all staff positions to any student in the Department of Home Economics and the School of Agriculture. At the same time the students in Home Economics decided to place their fullest efforts behind the Farmer instead of trying to start another publication on the campus.

This matter is of importance since the Home Economics students assumed that the Homemaker section was a separate publication in the Farmer and that they were not eligible for the main staff positions. No ruling had ever been made to that effect. but that was the common belief.

We, therefore, urge all Home Economics students interested in publication work who come out for the staff to try for the top positions of editor-in-chief or business manager since all staff positions are gained on a competitive basis.

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Many types, sizes, prices, etc. Larges line of ELECTRIC poultry supplies i U. S. Eastern Brooder stocks. Prove and economical incubators, heaters, thermostats, alarms, insect electrocutors, etc. Free catalog.
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Send for FREE Catalog. Also lists Wire Fabrics and Pen Materials for Poultrymen.
Ask for New Circulars on Laying Batteries
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ONLY $17.50
Parts for Batteries of every description including Laying Cages

"I'm adding alfalfa to the rotation this spring, Bill."
"That's great! It's the thing, but you may need to do some liming."

SERVING FARMERS OVER A QUARTER CENTURY

OVER THE FENCE

J. B. R. Dickey of State College says: "Satisfactory legumes and pasture cannot be produced on sour or lime-deficient land. With a few exceptions, Pennsylvania soils are naturally sour, or soon become so as the lime is removed in crops or leached out in drainage water. This lime must be replaced if livestock is to be well and cheaply fed, and the soil kept productive. Soil acidity is the most common single cause for the failure of legumes to thrive and withstand winter killing. The substitution of alfalfa for clover has been increasing rapidly. Alfalfa requires more lime and more intelligent liming than other legumes."

Correct soil acidity for profitable yields with Carbon pulverized and ground, kiln-dried limestone, available for immediate delivery. See your local Carbon Limestone Dealer.

Quarry located at Hillsville, Pa.

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Animal Disease Research Laboratory

by W. T. S. Thorp

WE are all aware of the toll diseases of domestic animals take from the yearly income of the farmer. This loss is true, regardless of the type of animal production in which one is most interested. Disease, however, may appear to be more of a problem in some animals than in others, but over a period of several years one will find very little difference when the value per head and the percentage loss is taken into consideration.

One has only to go back a few years in the history of the livestock industry to determine the benefits derived from animal disease research. The control of such diseases as tuberculosis in cattle, swine, and poultry has all been based on disease research over a period of time. The same is true of Texas Fever, Hog Cholera, Bang's Disease, Pullorum Disease, and many others. In all of the research on the above diseases certain fundamentals have necessarily preceded any control measures. A number of such fundamentals have come from the medical profession in general and from closely related sciences such as bacteriology and chemistry.

It is the plan of the laboratory at the Pennsylvania State College to confine its efforts entirely to disease research on those problems which are most widespread and of paramount importance to Animal Husbandry in general. This will be greatly facilitated since an excellent diagnosic laboratory for routine diagnosis is maintained by the Bureau of Animal Industry at Harrisburg. A good start has been made in equipping the laboratory for its various research problems although certain phases will be somewhat limited for a short time until further equipment is added. The disease research is in close cooperation with the various departments of the School of Agriculture.

A number of other institutions and the United States Bureau of Animal Industry are working on the diseases of dairy cattle, especially Bang's disease, and Veneral Trichomoniasis. A smaller number, however, are doing a limited amount of research on diseases of growing calves. The chief diseases encountered in raising young calves to mature separately or as a combination of both conditions. Since September 1938, research has been instigated on both of these diseases. Some important information has been obtained in regard to the causative agents and nature of the disease. These problems are receiving considerable attention in the laboratory. In addition to the above, pathological studies are made on all materials submitted from calves on nutritional experiments, especially those on Vitamin A deficient diets.

All poultrymen are aware of the losses to the poultry industry through diseases of infections or nutritional causes. Since the poultry industry of Pennsylvania is quoted as being second only to dairying in value and since the disease problem is of such magnitude, a major portion of the efforts of the laboratory is spent on research work in poultry diseases. Pullorum disease, which took its toll not so long ago, has for the most part reached the stage of control by regular testing and proper sanitary measures. The results of this work are directly proportional to the quality of the test which is used. That complex group of diseases commonly known as "Fowl Paralysis" is still one of the most, if not the most, important disease problem to the poultrymen. The above statement is exemplified by the fact that practically the entire efforts of the new regional laboratory for Poultry Disease Research, located in Michigan, will be spent on "Fowl Paralysis". Our research at the Pennsylvania State College is in close cooperation with the regional laboratory. Considerable progress has been made from a breeding standpoint by the Department of Poultry Husbandry in regard to the paralysis problem. In addition to the Fowl Paralysis research, a study has been started on those diseases affecting young turkey poults. The most important of these is a septicemia resulting from an infection by Salmonella Aertrycke, an organism of the same type as Salmonella Pullorum which is the cause of white diarrhea in chickens.

The growth of the sheep and swine industry in Pennsylvania has become and is becoming of greater importance from year to year. At the present time various phases of the nutritional diseases of sheep and swine are being studied from a pathological viewpoint.

It is hoped that through the research as outlined above some additional contribution can be made to the control and prevention of animal diseases.

The Horse

(Continued from page 197)

therefore much below the efficiency while working, and it varies with the number of working hours of each day. Efficiency decreases with increasing hours of work each day.

The variation of profit with hours worked each day gives a different result. From this angle, unlike efficiency, profit is linearly related to the number of hours worked; that is, the increase in profit each day is constant.

The gross efficiency of horses approaches 25 per cent regardless of weight. There is no difference in the 1000 lb. or 1800 lb. horse on efficiency basis.

Under average farm conditions, the maximum overall efficiency when he works but 8 hours each day at the rate of one horse power is about 14 per cent. This 14 per cent efficiency includes the energy cost of maintenance during the 16 hours rest. In the tractor there is no energy expense during hours when not used, although the horse is much more efficient energetically than the farm tractor while working. The all-day energy efficiency of the horse is the same order as the overall efficiency of the tractor while working.

Efficiencies of Motors

Steam Engines without condensers—7½ per cent.
Steam Engines with condensers—9 to 19 per cent.
Gas Engines—14 to 18 per cent.
Diesel Engines—29 to 35 per cent.

A ten year study on 57 farms re-

(Continued on page 221)

OUR DEBT TO EVAN PUGH

by R. Adams Dutcher

In 1859 Dr. Pugh accepted the invitation tendered by the President of the Board, Frederick Watts. He was given $1000 to purchase equipment and apparatus in Europe. He arrived in State College in September, 1859, but did not actually assume his duties until February, 1860, when he delivered the Inaugural address on the "Mutual Relations of Teacher and Taught."

In addition to his German university experience he had visited for a time in France where he made a study of trade schools and trade school curricula and throughout his short administrative life, the influences of these European observations are continuously making themselves manifest.

Pugh was faced with a multitude of problems when he began his Presidency in October, 1859. Courses of study had to be organized; rules and regulations regarding conduct and discipline had to be created; the people of the Commonwealth had no idea of his aims and objectives—hence it became necessary to educate the public and the legislature to the importance of the new educational undertaking. Old Main was not yet completed and funds had to be found to put the physical plant in condition for satisfactory class work. The Federal Land Grant Act had not yet been passed and it was necessary for Pugh to use much of his time and energy combating open opposition and criticism for this new fangled idea that was bound to cost the people more money. Naturally, the plan was not understood by the educational leaders of the day and, futhermore, it was but natural that considerable opposition should come from privately endowed denominational colleges.

Dr. Runkle points out that Pugh as a teacher did not occupy the chair of chemistry or of science. His was a settee of subjects. In addition to his presidential duties, he was Director of Chemical Laboratories and Museum, Dean of the Faculty, Publicity Director, Superintendent of Grounds and Buildings, Purchasing Agent, Dean of Graduate Instruction, Dean of Men, Director of Athletics and Farm Superintendent.

With all of these duties he had not forgotten research. It is recorded that he had two graduate students working on research problems in 1862 and in the following year the number had increased to eleven. You can see, therefore, that the term Farmers High School was not used in the sense that we use it today. It was really comparable to the German Hochschule which is similar to our technical college.

In speaking of our debt to Evan Pugh I can do no better than quote from Dr. Runkle's papers: "A carefully prepared address was delivered at Carlisle in 1860, and later printed, in which Pugh blocks out the needs of Pennsylvania, agriculturally. Secondary Schools of Agriculture headed by our own developing Agricultural College is of prime importance. A State Chemist and division of Agricultural statistics are urged, also a National Bureau of Agriculture. More significant than all is his insistent anticipation of Agricultural Scientific Investigation Stations, whose work he outlines as: (1) To establish by experiment the facts and principles to be taught; (2) To establish an experimental farm; (3) To install laboratories for analysis; (4) To equip stalls, stables, etc., for experiments on feeding and for determining the comparative value of foods; (5) To issue a paper or publication to put results into the hands of the farming profession. Here are instruction, research and extension, with emphasis, too, of animal nutrition and physiology, as the mansard roof of the Agricultural temple. He wrote a concise history of Agricultural Education in Europe, including the Farmers' High School and Agricultural College to 1862. "A Report on Organization and Facilities for Colleges of Agriculture and Mechanic Arts with special reference to the Agricultural College of Pennsylvania in view of its endowment by the Land Scrip Fund," printed January 6, 1864, is one of the most important of Dr. Pugh's papers. It is worthy of study today, not only as an index of the comprehensive grasp of his mind, but for its prophetic reach as foreshadowing the history and genius of these Industrial Institutions. He assumes as self-evident the need of investigation, laboratories, models, machines, museums of raw materials and exhibits of processes of manufacture, and he everywhere points the moral by illustrations from institutions studied by him in Germany, France and England. Courses of study are evaluated; and his faculty, a minimum for an Industrial College, is twenty-nine. The Pennsylvania Agricultural College had in 1864, five Professors, one Assistant Professor and one Lecturer."

Time does not permit me to enumerate the professorships and courses he prescribed as necessary for the new type of college but let it suffice to say Agricultural College Curricula today are but refinements of his original plan.

As soon as the Land Grant Act was passed, political pressure came from every source to dissipate the funds by diverting them to other types of educational institutions, by establishing Agricultural Professorships in so-called trade schools and colleges, etc. One of our greatest debts to Pugh lies in the fact that he played a major role in preventing this catastrophe. He made many trips to Washington in the interest of this work.

He was offered a number of lucrative Federal positions paying more money than he received as President of the Farmers' High School. While some of these offers were very tempting, he wrote Samuel W. Johnson that his mind was made up. He was going to stay and set his pet project through. I might add that, as President, he received the enormous salary of $1500 per year. Out of this he donated $500 per year for laboratory equipment.

Through his efforts Old Main was completed and his chemistry laboratories were considered second to none. He made the State "Agricultural College conscious" and recommended that the name of the institution be changed to "The Agricultural College of Pennsylvania." While the Civil War undoubtedly affected student attendance, there were 142 students en-

(Continued on page 216)

On the Trail of Tender Meat

(Continued from page 198)

made prime by tenderizing. It was tender but still tasted medium.

Other Tenderizing Methods

The housewife can tenderize a steak by making a marinade, consisting of the juice of a lemon and two tablespoons of olive oil mixed and spread over both surfaces of a steak. Place the steak in an earthen dish and set in a refrigerator for several days. The acetic acid in the lemon juice will tenderize the meat and the olive oil will exclude the air and hold down off flavors.

Roasts can be tenderized by placing them overnight in a weak vinegar solution.

Retailers tenderize steak by the use of cubing machines or waffling machines or by scoring.

Meat may also be tenderized by the use of the powerful enzymes papain or bromelin which are spread over meat in a liquid form and tenderize by breaking down the proteins. These are applied about five minutes before cooking, after which the heat will then destroy the enzyme and end the process.

Dairy Manufacturers Take Trip

The Seniors of Dairy Manufacturing left for their spring field trip Sunday April 2. This field trip is an annual affair and the itinerary was organized by Professor C. D. Dahle for twenty-two seniors in dairy manufacturing. The trip included the two large milk and ice cream consuming cities of the East; namely, Philadelphia and New York city.

The group visited such plants as Supplee-Wills-Jones Milk Co., Supplee-Wills-Jones Ice Cream Co., Abbotts Milk Co., and Breyers Ice Cream Co. all of Philadelphia and Walker-Gordon Co., Plainsboro, New Jersey. During their stay in the largest milk consuming city in the United States they visited Breyers Ice Cream Plant and Reid Ice Cream Plant. While in New York they enrolled in the Ice Cream Merchandizing Short Course which was given by the International Association of Ice Cream Manufacturers, Washington, D. C.

SHORTHORN CATTLE
The popular farm cattle

SHORTHORNS are experiencing an enlarged outlet for crossing purposes on the range. The establishing of small herds in farming areas is definitely on the up.

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BOTH RIGHTS AND LEFTS

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AND OF COURSE . . . AGRICULTURAL BOOKS ON ALL SUBJECTS

***Shop at* METZGER'S** Penn State 1915

Debt to Pugh

(Continued from page 200)

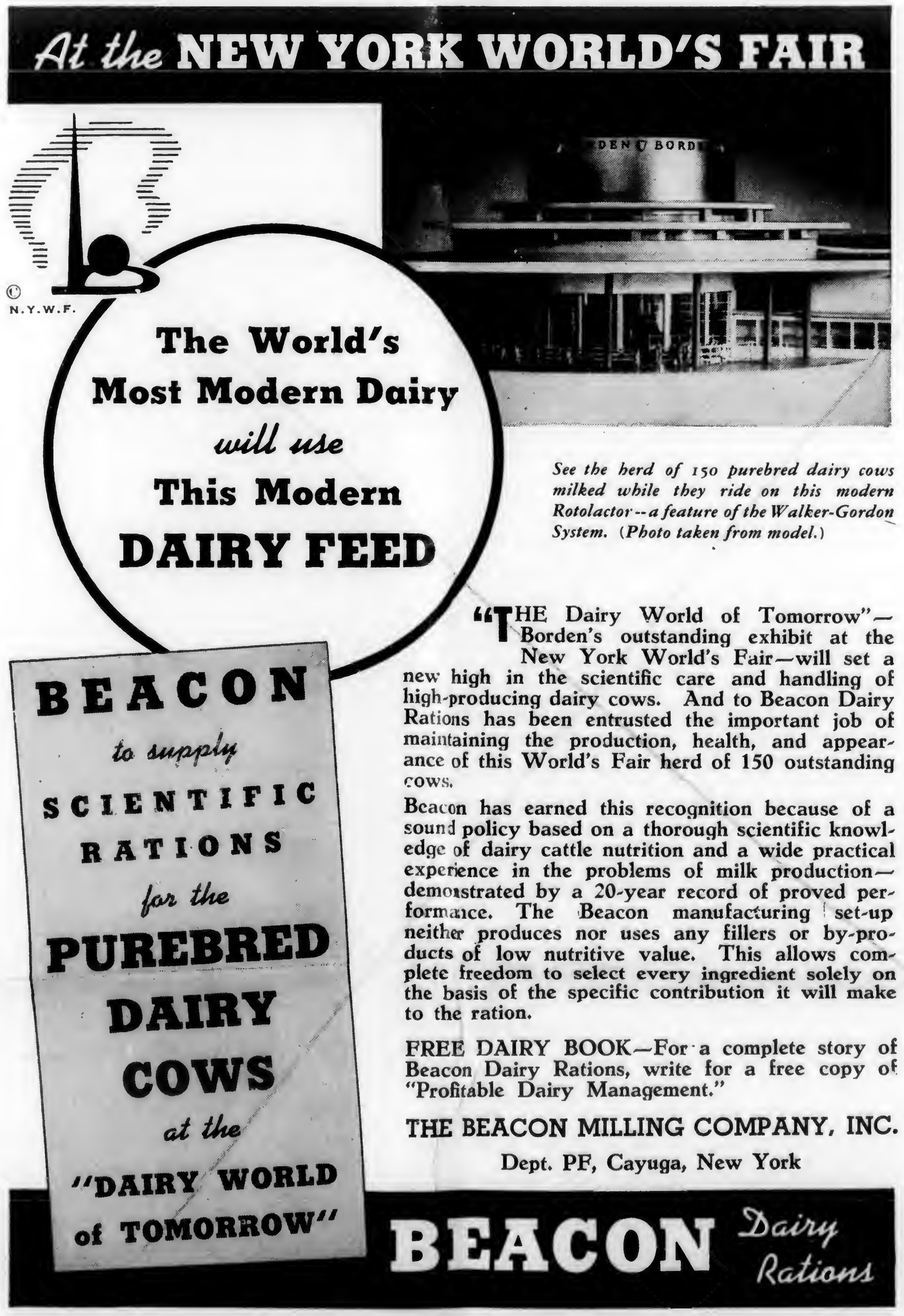
rolled in 1863 and 146 in 1864. I have already mentioned the fact that eleven graduate students were enrolled in 1863. These topics covered the fields of Agriculture, Chemistry, Botany and Geology.

It is clear that his abilities were appreciated since the Editor of the Philadelphia Ledger stated as early as 1859 that "there are not five men in the Commonwealth whose ability would not be taxed by the headship of the Institution."

He offered to advance $1000 of his own money toward the construction of a President's home if the State would furnish an additional $2000. The house was not finished when he died of typhoid fever on April 29, 1864. He had married Miss Rebecca Valentine of Bellefonte but two months prior to his death.

In conclusion may I repeat that no single individual has done as much to make Penn State what she is today. He was a great teacher, an outstanding scientist and a capable administrator. He sacrificed his life in an almost superhuman effort to lay the foundation of a great institution with high cultural and scientific standards.

While Penn State will always be the best possible memorial to the memory of Dr. Evan Pugh, let us hope that the time is not far off when the institution itself will express its appreciation and gratitude by some proper form of recognition. I hoped that the administration would consider the possibility of a suitable memorial during the present building program.



At the NEW YORK WORLD'S FAIR

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The World's Most Modern Dairy will use This Modern DAIRY FEED

See the herd of 150 purebred dairy cows milked while they ride on this modern Rotolactor--a feature of the Walker-Gordon System. (Photo taken from model.)

BEACON to supply SCIENTIFIC RATIONS for the PUREBRED DAIRY COWS at the "DAIRY WORLD of TOMORROW"

"THE Dairy World of Tomorrow"—Borden's outstanding exhibit at the New York World's Fair—will set a new high in the scientific care and handling of high-producing dairy cows. And to Beacon Dairy Rations has been entrusted the important job of maintaining the production, health, and appearance of this World's Fair herd of 150 outstanding cows.

Beacon has earned this recognition because of a sound policy based on a thorough scientific knowledge of dairy cattle nutrition and a wide practical experience in the problems of milk production—demonstrated by a 20-year record of proved performance. The Beacon manufacturing set-up neither produces nor uses any fillers or by-products of low nutritive value. This allows complete freedom to select every ingredient solely on the basis of the specific contribution it will make to the ration.

FREE DAIRY BOOK—For a complete story of Beacon Dairy Rations, write for a free copy of "Profitable Dairy Management."

THE BEACON MILLING COMPANY, INC.
Dept. PF, Cayuga, New York

BEACON Dairy Rations

Alfalfa Molasses Silage

(Continued from page 194)

taken as conclusive evidence until several further trials are completed.

1. The steers receiving corn and cob meal and corn silage with no dry roughage had a low calcium intake and it was necessary to furnish additional calcium in the form of ground limestone or steamed bone meal to keep them on feed.

2. The steers fed alfalfa molasses silage consumed almost twice as much silage as the corn silage lot and were not fed protein or mineral supplement.

Lots	Average Daily Gain	Cost of 100 lbs. Gain
I	2.08 lbs.	$9.18
II	2.36 lbs.	$8.50
III	2.13 lbs.	$9.52

Information About School

(Continued from page 202)

the remaining two, one has chosen extension work and the other has selected marriage.

The girls who studied Institutional Administration have entered various fields. Nine of them are furthering their education by taking their dietary interneships. Two are nutritional advisers with the Farm Security, while four more are commercial demonstrators for food, electrical or other utility companies. One is with a school cafeteria and another is a Y. M. C. A. dietitian. Marriage claimed two from the group.

Because the girls from Option III are engaged in such varied types of work, the employment records are not available.

If one studies these statistics, surely, she will know more about her College and she will have a better idea as to what she may expect after graduation.

Reprinted from JOURNAL OF DAIRY SCIENCE, May, 1939, Vol. XXII, No. 5, pages 321–328.

CAROTENE IN THE NUTRITION OF DAIRY CALVES*

C. C. FLORA, R. E. WARD AND S. I. BECHDEL
Department of Dairy Husbandry
AND
N. B. GUERRANT AND R. A. DUTCHER
Department of Agricultural and Biological Chemistry, The Pennsylvania State College

The necessity of vitamin A for proper growth and well being of dairy calves has been conceded quite generally for the past ten years. It has been shown that for this species the general symptoms of vitamin A deficiency are slow growth, general unthriftiness, scours, blindness, susceptibility to pneumonia, and oedema.

Studies at the Pennsylvania Agricultural Experiment Station in which large amounts of cottonseed meal were fed to calves have shown definitely that supplements of cod liver oil or alfalfa hay of good quality will prevent the usual malnutrition or cottonseed meal injury that results from such feeding practice. If the injury had not progressed too far cures were affected by the same treatment. The vitamin A present in the cod liver oil and the carotene (the mother substance of vitamin A) in alfalfa undoubtedly played a major rôle in the prevention and cure of this malnutrition. Carotene dissolved in cottonseed oil, on the other hand, apparently had little or no effect in the prevention or cure of this nutritional ailment.

Jones, Eckles and Palmer (11) showed very conclusively that vitamin A is indispensable in the growth of dairy calves. Bechdel, Eckles and Palmer (3) in the same year reported that calves developed symptoms of vitamin A deficiency when cod liver oil was not included in their experimental ration of corn gluten feed, casein, polished rice and butterfat.

Converse and Meigs (6) found that cows fed on timothy hay of low grade for a period of two or more years were unable to produce normal calves. Calves fed on the milk from these cows soon died. The investigators found that vitamin A in the form of carotene or cod liver oil can be added to the deficient milk rations of the calves and by doing so make them adequate for producing approximately normal growth.

After depleting calves of their body store of vitamin A by feeding a basal diet composed of skimmilk, cottonseed meal, dried beet pulp and bone meal, Kuhlman, Gallup and Weaver (12) found that 5 ml. of a commercial carotene preparation fed in combination with a vitamin D supplement was as effective in relieving the vitamin A deficiency symptoms as 30 ml. of cod liver oil.

Received for publication, December 2, 1938.

* Authorized for publication on November 29, 1938, as paper No. 869 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

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2. The steers fed alfalfa molasses silage consumed almost twice as much silage as the corn silage lot and were not fed protein or mineral supplement.

Lots	Average Daily Gain	Cost of 100 lbs. Gain
I	2.08 lbs.	$9.18
II	2.36 lbs.	$8.50
III	2.13 lbs.	$9.52

Information About School

(Continued from page 202)

the remaining two, one has chosen extension work and the other has selected marriage.

The girls who studied Institutional Administration have entered various fields. Nine of them are furthering their education by taking their dietary interneships. Two are nutritional advisers with the Farm Security, while four more are commercial demonstrators for food, electrical or other utility companies. One is with a

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CAROTENE IN THE NUTRITION OF DAIRY CALVES*

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The necessity of vitamin A for proper growth and well being of dairy calves has been conceded quite generally for the past ten years. It has been shown that for this species the general symptoms of vitamin A deficiency are slow growth, general unthriftiness, scours, blindness, susceptibility to pneumonia, and oedema.

Studies at the Pennsylvania Agricultural Experiment Station in which large amounts of cottonseed meal were fed to calves have shown definitely that supplements of cod liver oil or alfalfa hay of good quality will prevent the usual malnutrition or cottonseed meal injury that results from such feeding practice. If the injury had not progressed too far cures were affected by the same treatment. The vitamin A present in the cod liver oil and the carotene (the mother substance of vitamin A) in alfalfa undoubtedly played a major rôle in the prevention and cure of this malnutrition. Carotene dissolved in cottonseed oil, on the other hand, apparently had little or no effect in the prevention or cure of this nutritional ailment.

Jones, Eckles and Palmer (11) showed very conclusively that vitamin A is indispensable in the growth of dairy calves. Bechdel, Eckles and Palmer (3) in the same year reported that calves developed symptoms of vitamin A deficiency when cod liver oil was not included in their experimental ration of corn gluten feed, casein, polished rice and butterfat.

Converse and Meigs (6) found that cows fed on timothy hay of low grade for a period of two or more years were unable to produce normal calves. Calves fed on the milk from these cows soon died. The investigators found that vitamin A in the form of carotene or cod liver oil can be added to the deficient milk rations of the calves and by doing so make them adequate for producing approximately normal growth.

After depleting calves of their body store of vitamin A by feeding a basal diet composed of skimmilk, cottonseed meal, dried beet pulp and bone meal, Kuhlman, Gallup and Weaver (12) found that 5 ml. of a commercial carotene preparation fed in combination with a vitamin D supplement was as effective in relieving the vitamin A deficiency symptoms as 30 ml. of cod liver oil.

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Bechdel (2), and Bechdel, Williams and Skaggs (4) produced typical cottonseed meal injury in calves. The symptoms simulated vitamin A deficiency, only the injury seemed to be somewhat more severe in that occasional cases of convulsions were observed. Cod liver oil gave adequate protection against the injury and brought about rapid recovery, while carotene seemed to be less effective in protecting against the cottonseed meal injury.

Hart and Guilbert (9) observed vitamin A deficiency in cattle under range conditions. They report (10) that about twenty-nine micrograms of carotene in alfalfa hay per day per kilogram of body weight would prevent or cure vitamin A deficiency symptoms in cattle.

Investigators have shown that the type of solvent used is an important factor in determining the biological value of carotene. According to Dutcher, Harris, Hartzler and Guerrant (7) carotene dissolved in mineral oil cannot be utilized by the rat.

Moore, Huffman and Duncan (14) observed blindness in calves dropped by cows while receiving roughage of poor quality. These investigators observed a similar condition among growing heifers fed the same type of ration. Kuhlman, Gallup and Weaver (13) reported two types of blindness among calves which may be related to vitamin A deficiency.

EXPERIMENT I

Grade Holstein calves were used as experimental subjects. The concentrate mixture fed was similar to calf rations commonly used on good dairy farms. In order to keep the vitamin A potency of the ration quite low, yellow corn was not included in the mixture. The constituents were as follows:

Wheat bran	150 pounds
Oat mids	200 pounds
Hominy feed	300 pounds
Linseed meal	100 pounds
Distillers' grain	50 pounds
Irradiated yeast	1 pound
Salt	2 pounds

The treatment of the calves previous to going on experiment consisted in feeding them whole milk until twenty days of age. At this time the whole milk was gradually replaced with skimmilk which was fed to all calves continuously while on experiment. Timothy hay of very low grade, almost devoid of green color, was fed prior to as well as in the experimental period. The skimmilk was produced by cows fed on a low vitamin A diet.

All animals were put on the basal ration at the beginning of the experiment, and no change was made until symptoms of vitamin A deficiency were observed. The symptoms first noticed were loss of appetite and scouring. This was followed by a general drop in body weight and blindness developed in two of the five animals used. The weight of the animal was the main criterion used in determining when to start supplementing the ration with the treatment.

A preparation of carotene dissolved in cottonseed oil was used as the source of vitamin A for two animals (calves 338 and 340). This preparation contained approximately 3,000 U.S.P. units of vitamin A activity per gram. The amounts of this supplement fed are given in Figure 1. It was fed daily by adding it to the skimmilk just before feeding. Two animals (calves 337 and 341) were fed two pounds of fresh carrots daily containing approximately 30,000 vitamin A units. Dehydrated alfalfa hay of good quality was fed to one calf (calf 342).

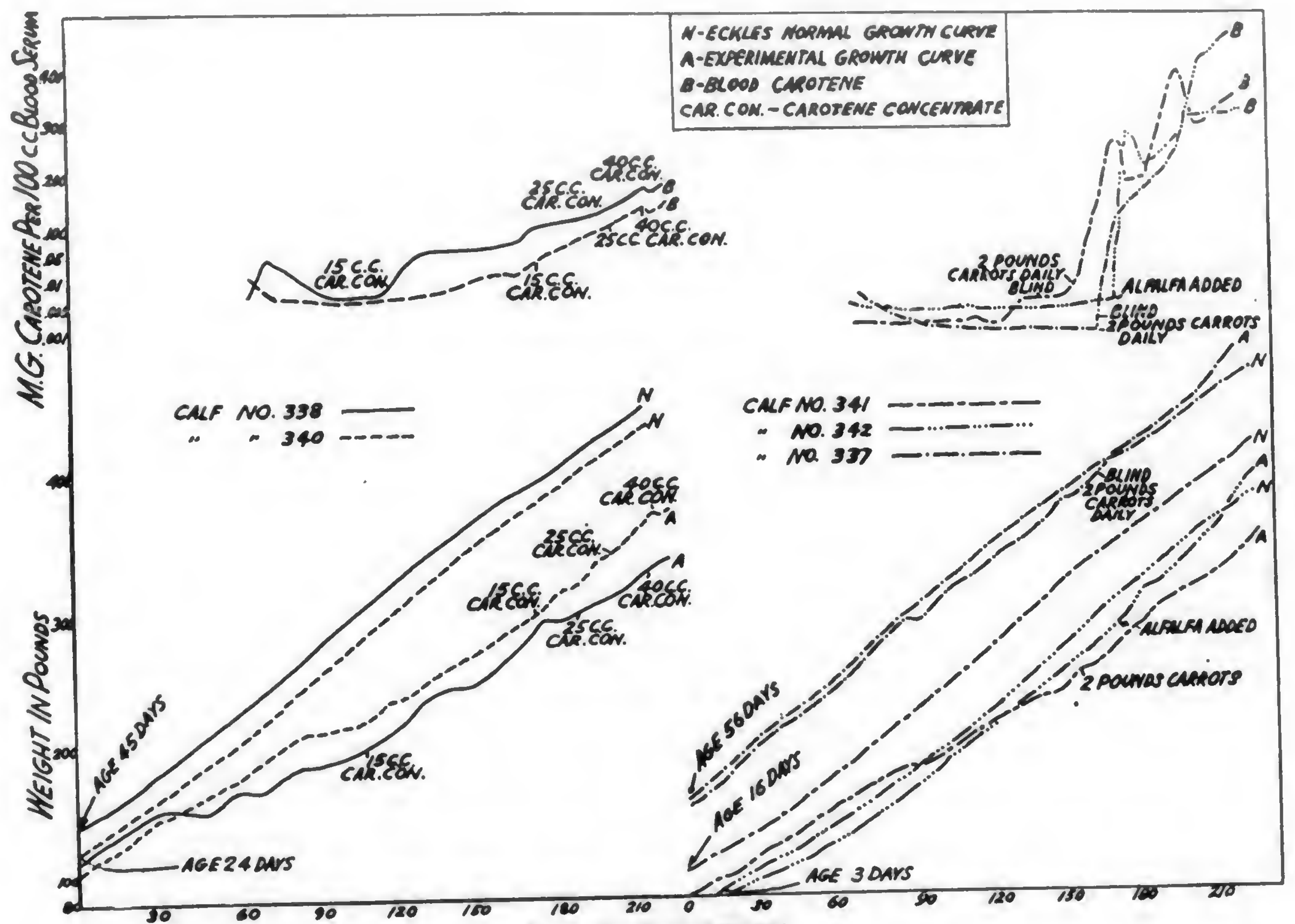

FIG. 1. Growth and blood carotene curves of calves used in Experiment I.

Carotene content of the blood serum was determined at regular intervals throughout the experimental period. The Palmer method modified by Conner (5), and White and Gordon (15) was used. Non-protein nitrogen, glucose and hemoglobin were determined on the blood of each animal at regular intervals.

RESULTS ON INDIVIDUAL CALVES

After the animals were depleted of their vitamin A reserve they were treated as individuals and no effort was made to experiment with them as groups or pairs.

The treatment and response of each animal is portrayed in Figure 1. The Eckles normal growth curve is used as a standard for growth.

Calf No. 338 which had received the carotene concentrate made normal growth for only the first 30 days on the experimental ration. It developed pneumonia on the 44th day and from that date on showed typical symptoms of vitamin A deficiency. Carotene supplement in large doses was administered on the 109th day and continued until the end of the experiment. This amount of carotene was effective in increasing the blood carotene slightly but the animal's growth rate gradually declined.

Calf No. 340 which also received the carotene concentrate made normal growth on the experimental ration for the first 85 days when typical symptoms of vitamin A deficiency, scours, loss of appetite and nasal discharges appeared. The administration of the carotene supplement did not increase the blood carotene or the growth rate to an appreciable extent.

Calf No. 337 made normal growth on the experimental ration for the first 81 days. It showed typical vitamin A deficiency symptoms, consisting of scours, loss of appetite and finally blindness on the 164th day. When carrots were fed, the blood carotene increased rapidly and the growth rate was accelerated.

Calf No. 341 was below normal in weight when put on experiment. Its growth rate remained almost constant for the first 80 days, but declined appreciably from this time on until 156 days on experiment. Blindness was observed on the 142nd day. About one week after carrots were added to the ration the growth rate began to increase. A phenomenal increase of the blood carotene was also observed after carrots were fed.

Calf No. 342 made practically normal growth gains although she showed symptoms of vitamin A deficiency such as scours, loss of appetite and nasal discharges. The addition of alfalfa to the ration increased the blood carotene immediately, and the rate of growth was also increased remarkably. Other symptoms of vitamin A deficiency also disappeared promptly.

GENERAL DISCUSSION

The results with calves Nos. 338 and 340 indicate that carotene dissolved in cottonseed oil is not utilized to the extent that it will prevent or cure avitaminosis A that results from feeding the above experimental rations. There is evidence that the calves utilized some of the carotene but not enough to make its effect significant.

Carrots were quite effective in correcting the avitaminosis A (blindness excepted) in calves Nos. 337 and 341. Alfalfa hay of good quality (dehydrated) appeared to be slightly more effective than carrots as shown by the results on calf No. 342. This is explainable through the fact that the alfalfa was fed in relatively large amounts and, therefore, more vitamin A units were supplied than in the carrots fed to the calves Nos. 337 and 341.

The blood carotene studies are of special interest in this connection. The chart reveals that the carotene appeared in the blood stream almost immediately in large amounts when carrots or alfalfa were fed to the calves suffering from avitaminosis A. The blood carotene was increased in amounts that were barely measurable when a carotene supplement was fed to the calves suffering with avitaminosis A. It is interesting to note, as indicated in the chart, that the potential units of vitamin A in the carotene supplement administered were much greater than the total unitage contained in the carrots.

Non-protein nitrogen, hemoglobin, and sugar determinations made during the depletion period did not show abnormal values as found by Anderson, Gayley and Pratt (1).

EXPERIMENT II

A second experiment was planned with the view of obtaining further information on the effectiveness of carotene in preventing and in curing the usual avitaminosis A that occurs from feeding calves on hay of poor quality. Timothy hay of low grade (with little green color) was used in this experiment. Six calves (calves 354, 356, 357, 361, 362, and 363) were fed a concentrate ration low in vitamin A. This grain ration was very similar to rations commonly used by dairy farmers. Five other calves (calves 349, 350, 355, 364, and 365) were fed a grain ration which contained fifty-seven per cent cottonseed meal. Two of these calves (calves 364 and 365) received no hay. All calves were left with their dams for two or three days after birth. They were then fed whole milk until two weeks of age, after which skimmilk was gradually substituted for the whole milk. All calves were weighed at weekly intervals at which time blood samples were taken for carotene determinations.

The timothy hay, assayed by the colorimetric method, was found to contain 4.5 micrograms of carotene per gram. The carotene supplement was guaranteed to have a biological activity of approximately 3,000 U.S.P. units of vitamin A per gram. This concentrate was weighed into $\frac{1}{4}$ and $\frac{1}{2}$ ounce capsules and administered by means of a balling gun. The livers of all calves that were slaughtered were assayed for vitamin A by the antimony trichloride method as outlined by Guilbert and Hart (8).

One of the calves (calf 362) receiving the low vitamin A ration was fed four and one-half grams of the carotene supplement beginning with the sixth week and continuing to the end of the experiment. The five other calves were fed the basal ration until marked symptoms of vitamin A deficiency occurred. At this time amounts ranging from four and one-half to eighteen grams of the carotene supplement were administered daily to the different individuals (Figure 2). Three of the calves (calves 349, 350, and 355) receiving the high cottonseed meal ration were treated in a like manner. One of the two calves (calf 364) receiving no hay was given four and one-half grams of the carotene supplement daily. The other calf (calf 365) was continued on the basal ration until it became very weak. Nine grams of the carotene supplement were then administered to this animal daily. Here again, the chief criterion for starting the administration of carotene supplement was failure of the animal to gain in weight. Other symptoms of vitamin A deficiency, especially dilated pupils and blindness, were used as further means of diagnosis.

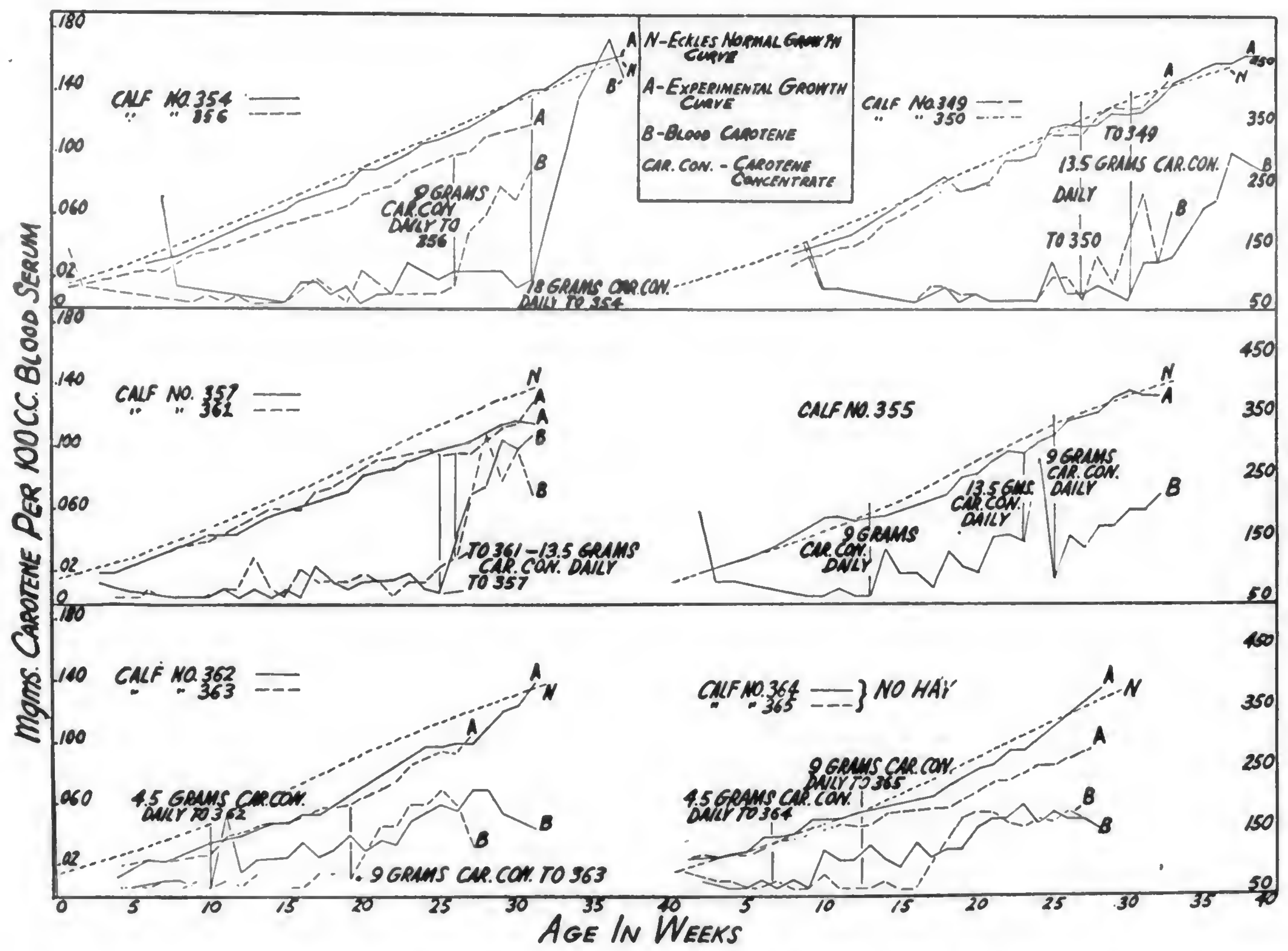

FIG. 2. Growth and blood carotene curves of calves used in second experiment.

RESULTS AND DISCUSSION

In every case following the administration of the carotene concentrate there was a marked improvement in general condition of the calf which also was usually accompanied by an accelerated growth rate. The effect of carotene administration on blood carotene varied from slight increases in some cases to very decided increases in other cases (Figure 2). The increases observed correspond roughly with the quantity of carotene administered. The relative amounts of vitamin A in the liver tissues also were found to correspond closely with the quantity of carotene supplement administered. This fact offers further proof that the carotene supplement was being utilized by the calves as a source of vitamin A.

The calves fed on the high cottonseed meal ration (calves 349, 350, 355, 364, and 365) exhibited symptoms which corresponded closely with the symptoms exhibited by those on the low vitamin A ration without cottonseed meal. They responded to the administration of the carotene concentrate in the usual manner. The calves on this ration showed a greater tendency toward intestinal disturbances and they also exhibited symptoms of vitamin A deficiency in a much shorter time than did similar calves receiving the other low vitamin A ration which contained no cottonseed meal.

The poor response of calf No. 365 while receiving no hay indicates the importance of hay as a source of carotene in the nutrition of calves. In this case distinct symptoms of malnutrition were produced very readily in the absence of both hay and carotene from its ration. Following the administration of 9 grams of the carotene supplement daily this animal showed marked improvement but never became normal. On the other hand, four and one-half grams of the carotene supplement when added to the ration of calf No. 364, was sufficient to prevent malnutrition in this animal for a considerable period (Figure 2).

Blindness was not observed in those calves in which severe avitaminosis A appeared quite rapidly. It appeared, however, in cases of those which had undergone a prolonged period of mild avitaminosis A during which the growth rate was only slightly subnormal. By carefully examining the eyes of the animals, it was found that the pupils were always dilated, and that the onset of blindness could be detected.

The carotene supplement used in this experiment, when administered in capsules and used in combination with a ration which was adequate in other respects, appeared to give results which compare very favorably with the results that had been obtained previously with alfalfa, carrots, and cod liver oil. The more clear cut and significant responses obtained in the second experiment as the result of feeding vitamin A deficient calves definite amounts of the carotene concentrate are in all probability due to improvements in technic and to more extensive data obtained through the use of a larger number of animals.

SUMMARY

Avitaminosis A was produced in growing calves through the use of poor quality bleached and aged timothy hays fed in connection with a grain mixture. Fresh carrots and alfalfa hay (dehydrated) of good quality were quite effective in correcting this avitaminosis A in calves. A commercial carotene concentrate also proved effective in preventing and curing the avitaminosis A that resulted from feeding calves rations of low carotene content, as well as overcoming the malnutrition resulting from feeding high percentages of cottonseed meal.

ACKNOWLEDGMENT

The carotene preparation used in the experiments was supplied by the Ralston Purina Company, St. Louis, Missouri.

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Vol. 17, No. 5, May, 1939

THE INFLUENCE OF EXERCISE ON THE GROWING RAT IN THE PRESENCE AND ABSENCE OF VITAMIN A[1, 2]

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FIVE FIGURES

(Received for publication January 9, 1939)

Research literature in the field of nutrition abounds in unexplained variations and discrepancies owing to numerous biological variables which are difficult if not impossible to control. This is especially true of those data obtained in investigations dealing with various phases and forms of malnutrition. Investigators have long recognized certain influencing factors and have attempted to determine the relative magnitude of their respective influences on the ultimate experimental results. The importance of heredity and previous nutritional history of the experimental subjects, together with such factors as age, sex and size, have been rather extensively investigated. Other factors including radiant energy and environmental temperatures and humidities have also received consideration. Although it has been frequently assumed that a relationship exists between the physical activity of an experimental animal and its nutritional response, little actual experimental work has been done toward establishing the relative significance of this factor, and these efforts have been directed mainly from the standpoint of energy requirements.

[1] Authorized for publication December 9, 1938, as paper no. 876 in the journal series of the Pennsylvania Agricultural Experiment Station.

[2] Presented before the division of biological chemistry at the Milwaukee meeting of the American Chemical Society, September 5 to 9, 1938.

It appears that adequate investigations have not been conducted regarding either the relationship of physical activity to the vitamin requirement of various species of animals or to the effect of vitamin intake on the inclination of such animals to exercise. Yoshiue ('22) reported that vitamin B deficient rats which were exercised died before developing the muscular paralysis typical of this deficiency. Blacklock ('24), on the other hand, has expressed a belief that lack of exercise accelerates and accentuates the ill effects of diets deficient in vitamin B. This author cites the fact that the disease beri beri most frequently occurs in institutions and in those situations where a fixed diet and some degree of confinement are usually maintained. Keith and Mitchell ('23) placed rats receiving a vitamin A deficient diet in cylindrical boxes and rotated the boxes at the rate of thirteen revolutions per minute for periods ranging from 30 to 40 hours per week. As a result of the studies these authors concluded that exercise distinctly hastened the appearance of the symptoms of vitamin A deficiency and hastened the death of young animals receiving vitamin A deficient diets. Similar studies carried out by these authors while employing diets deficient in vitamin B did not show that the requirement for this dietary factor was affected by exercise. In addition to these citations, numerous authors have inferred that they recognized certain influences of exercise on vitamin requirement as well as increased physical activity as the result of adequate vitamin intake, although no data were given in support of such statements.

It would seem that if exercise does influence vitamin requirement some of the variations in individual response of experimental animals comprising a carefully selected group might be due to differences in physical activity of different animals in the group, and also that differences in responses obtained by different investigators might be at least partially explained on the basis of the difference in the degree of freedom allowed the experimental subjects. With these thoughts in mind it appeared that the influence of exercise or activity of the experimental animal on its vitamin requirements and also the effect of vitamin intake on physical activity merited further study. The present report is concerned primarily with the influence of exercise on the development and well being of the growing rat in the presence and absence of vitamin A. Similar studies are now under way with reference to other vitamins.

EXPERIMENTAL

The investigation consisted essentially of a comparison of the physiological activity and growth responses of comparable groups of young rats while being maintained under identical conditions with the exception of the amount of exercise permitted by the types of caging employed and of the vitamin A intake. The experimental equipment consisted of three sets of individual all-metal cages, of which two sets were of rotating type, having a wheel approximately 14 inches in diameter that was equipped with a registering cyclometer. One set of the rotating cages was so arranged on one side of a large table that the wheels could be rotated at a definite speed by means of a continuous belt driven by an electric motor through a gear reducing mechanism. The second set of rotating cages was arranged on the opposite side of the table and the wheel of each cage was adjusted so as to allow uniform freedom of rotation. The third set of cages consisted of our regular laboratory cages, each of which had approximately 120 square inches of floor space. All cages were provided with raised screen floors. Clean paper was kept under the cages.

Healthy rats, 21 to 23 days of age and weighing approximately 45 gm., were used as the experimental subjects. A special effort was made to place comparable animals of the same litter, sex and body weight in the three respective groups. All animals were fed ad libitum a vitamin A deficient basal diet which consisted of: purified casein 18, salt mixture 3, Cell-U-flour 2, dextrinized cornstarch 77 and irradiated yeast 8. Clean distilled water was available at all times.

Each set of animals was subdivided into two groups. Animals of one group received only the vitamin A deficient diet until depleted of their body reserve of this vitamin, while the animals of the second group served as positive controls and in consequence each received 2 U.S.P. units of vitamin A daily. After being depleted, the animals of the former group also received this quantity of vitamin A daily for a period of 35 days.

The animals of the forced exercise group were allowed access to the rotating wheel only during the period of rotation. During the remainder of the time they were confined in the small side cage (60 square inches of floor space) containing the food and water. The animals of the voluntary exercise group and those housed in the stationary cages were allowed free access to the entire cage at all times.

Observations made on a group of normal young rats which received our breeding colony diet, housed in the above type of rotating cage and allowed freedom of exercise, had shown that an average of 1000 revolutions per day was made during a period of 70 days. This number of revolutions was taken, therefore, as the basis for the maximum daily rotation of the forced exercised groups. Previous observations had also indicated that a speed of approximately six revolutions per minute, with this particular type of cage, was the most effective in keeping the animals in constant motion. Speeds less than six revolutions per minute allowed the animals to stop frequently, while greater speeds tended to cause the animals to cling to the wheel and 'ride' instead of exercising as was desired. At the above rate of rotation, approximately 3 hours per day were required to rotate the cages of the forced exercise group 1000 revolutions or a distance of approximately 0.7 mile. This period of rotation was always started in the early forenoon following the weighing of the animals. At the end of the rotation period each animal was again returned to the small side cage containing the food and water where it remained until the following day.

A daily record was made of the weight of the animal, the amount of food consumed, the number of fecal pellets and the number of revolutions made by the wheels of the rotating cages during the previous 24 hours. All fecal matter was removed from the cage pans daily and stored in glass containers. At the end of each week the fecal collection from each rat was dried and weighed. Daily observations were also made of the animals in an effort to detect the onset as well as the relative stage of the symptoms characteristic of vitamin A deficiency. As the body weight of each animal ceased to increase and symptoms of xerophthalmia appeared in one or both eyes, the animal was given daily doses of 2 U.S.P. units of vitamin A as a supplement to the deficient diet (see above). The duration of the experimental periods for all animals ranged from 64 to 71 days, depending upon the time required to deplete the respective animals. Those animals receiving the vitamin A supplement from the beginning of the experiment were continued under observation for a period of 70 days.

DATA

In order to conserve space the data obtained for individual animals have been reduced to group averages and are presented in the following charts (see figs. 1 to 5 inclusive). Only data from those animals which survived the entire experimental period have been used in tabulating these summaries.

DISCUSSION

It was observed at the beginning of the experiment that those animals housed in the rotating cages and composing the forced exercise group were unable to undergo the full 1000 revolutions before becoming completely exhausted. In order to avoid any possible vitiating effect of over exhaustion on subsequent responses, these animals were exercised during the first few days of the experiment to the point of partial exhaustion and the number of revolutions was increased daily until the end of the first week at which time the cages were undergoing the desired 1000 revolutions daily.

It may be observed from the data presented in figure 1 that exercise, under the conditions of this experiment, had a measurable depressing effect on the rate of growth of young rats irrespective of the vitamin A intake. The effect of this factor on the rate of growth was, however, somewhat greater among those animals which had been previously depleted of their body store of vitamin A. These findings are further emphasized by the data presented in figure 2 relative to the effect of exercise on the total change in body weight during the entire experimental period. Here again the animals of the confined groups, both with and without vitamin A, made the greatest increases in body weight. No attempt was made to determine the chemical nature of the weight increases of the respective groups of animals.

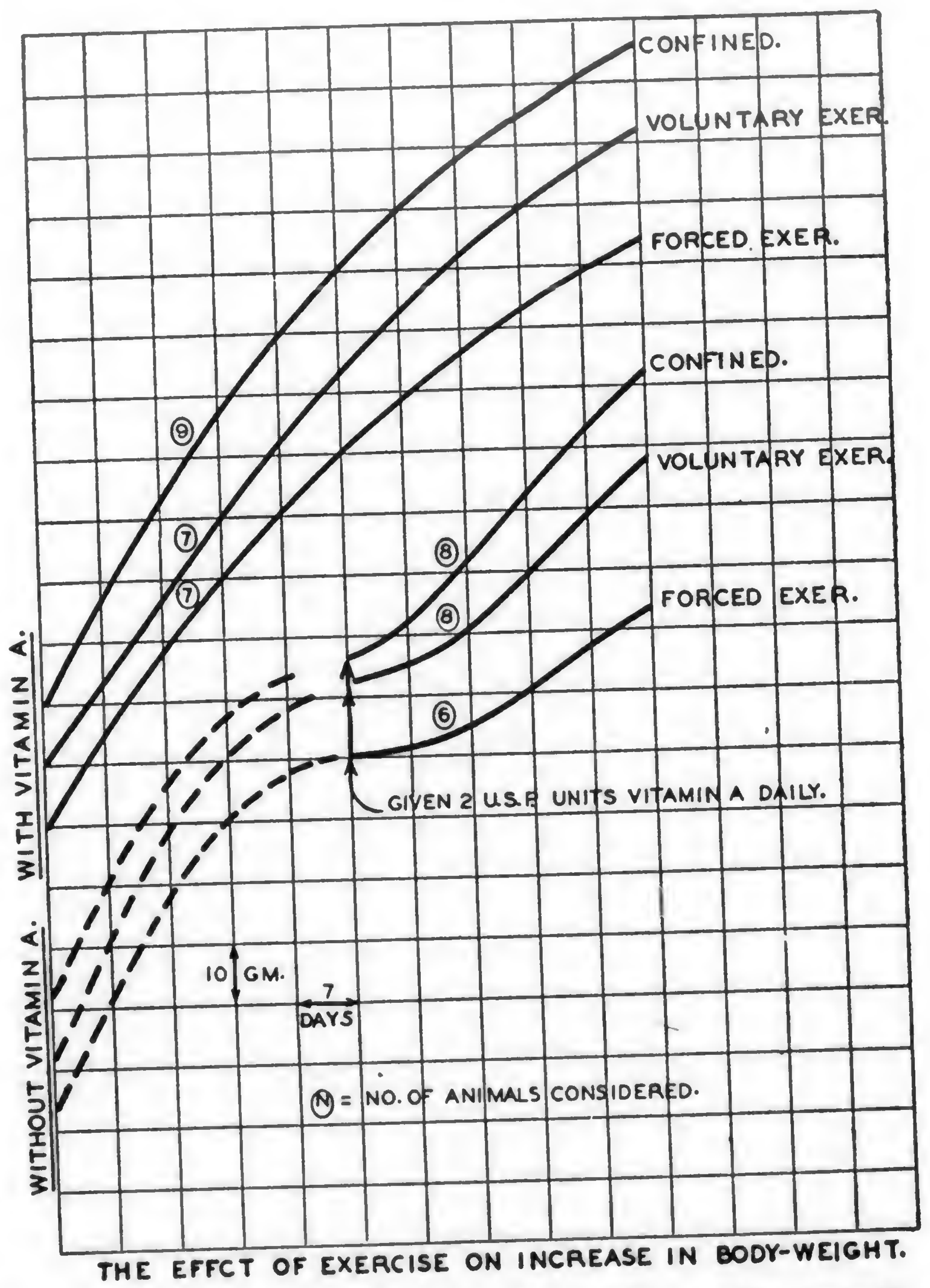

Fig. 1 The effect of different amounts of exercise on the growth rate of young rats while being maintained under comparable dietary conditions except as to their vitamin A intake.

The data presented in figure 2 concerning the total food intake of the various groups of animals are somewhat surprising in that it was expected that greater physical activity would result in greater food consumption in order to compensate for the greater energy exchange involved. Such is not indicated by the data obtained, for in each instance the group of animals undergoing the least amount of exercise consumed the greatest amount of food. Similar results have been reported by Keith and Mitchell ('23). While no explanation can be offered at this time for the suppressing effect of exercise on food intake, it seems highly probable that the physical effects of the 3 hours of rotation during the forenoon are important considerations.

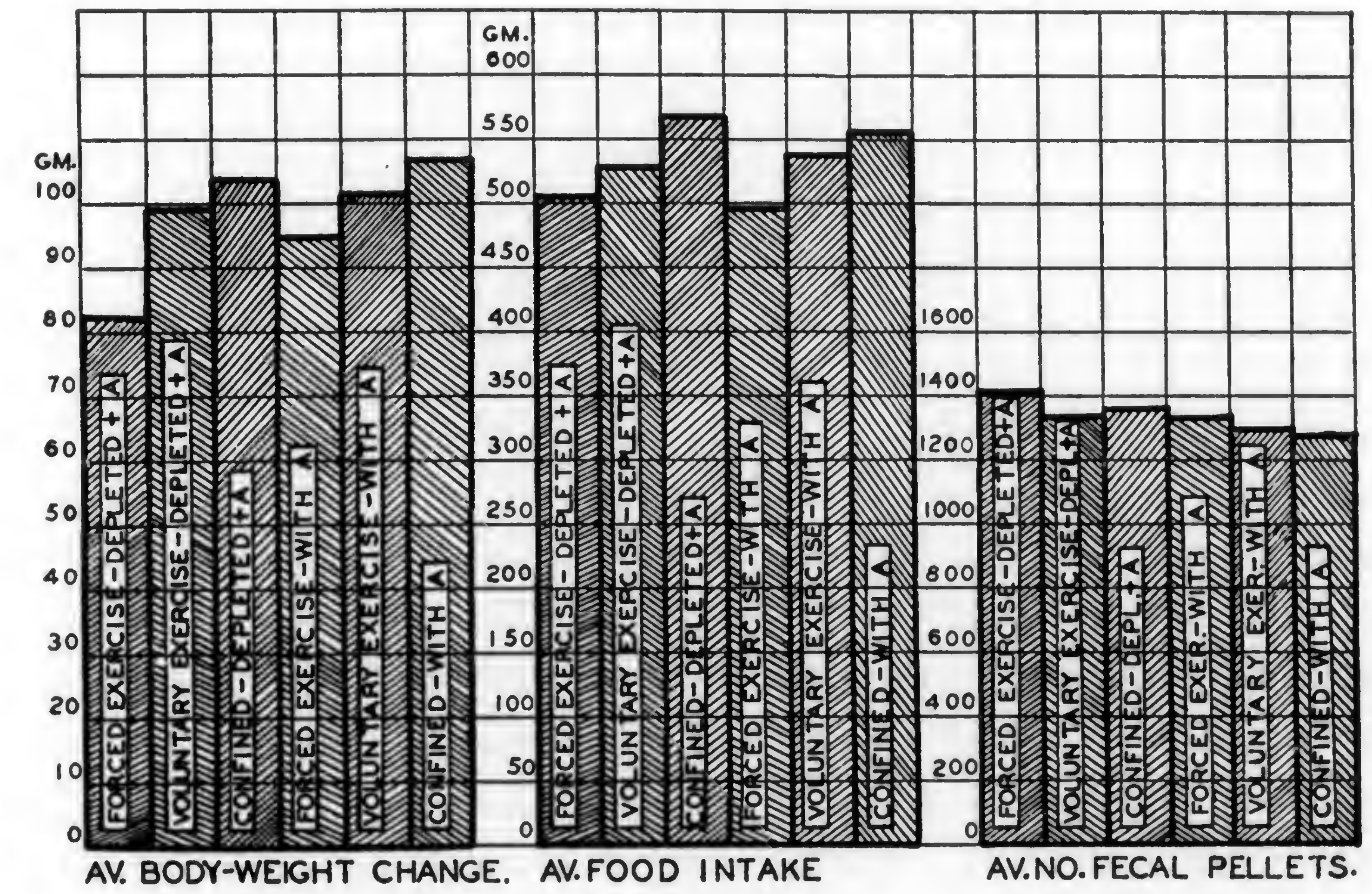

Fig. 2 The relative effect of exercise on the average body weight increase, average food intake and average number of fecal particles voided by young rats receiving a diet with and without vitamin A.

Another unexpected observation is the close similarity in the total amounts of food consumed by the animals of the corresponding groups irrespective of their vitamin A intake. These findings can at least be partially explained on the basis of the high food intake of the depleted animals during the 35 days the vitamin A supplement was fed.

Figure 2 also contains data concerning the effect of exercise on the average number of fecal pellets voided by the different groups of animals during the entire experimental period. It will be observed that while the number of fecal pellets voided by the different groups of rats did not vary widely, in each instance those animals composing the forced exercised groups voided the greatest number of pellets. These findings may be interpreted as signifying a favorable effect of exercise on intestinal motility and, in consequence, on elimination. Exercise is generally believed to have a similar effect on humans.

When one considers the total weight of the fecal matter, the relative proportions of food excreted as fecal matter and the average weight of the fecal pellets voided by the various groups of animals (fig. 3) differences between the groups become more significant. In all instances those animals of the confined groups voided the greatest weight of fecal matter, the greatest percentage of fecal matter and the heaviest fecal pellets. The lower percentage of fecal matter from the animals of the exercised groups might be explained on the basis that these animals were more efficient in the utilization of part or all of the constituents of their food than were comparable groups of animals which were not exercised. The smaller fecal pellets from the exercised animals further indicate increased intestinal motility among the animals of these groups.

From the data presented in figure 4 it may be observed that exercise has a significant effect on the time required for the cessation of growth and for the onset of xerophthalmia among rats receiving a diet deficient in vitamin A. The values given in these graphs represent the averages for all animals of the respective groups. It may be seen that it required, on

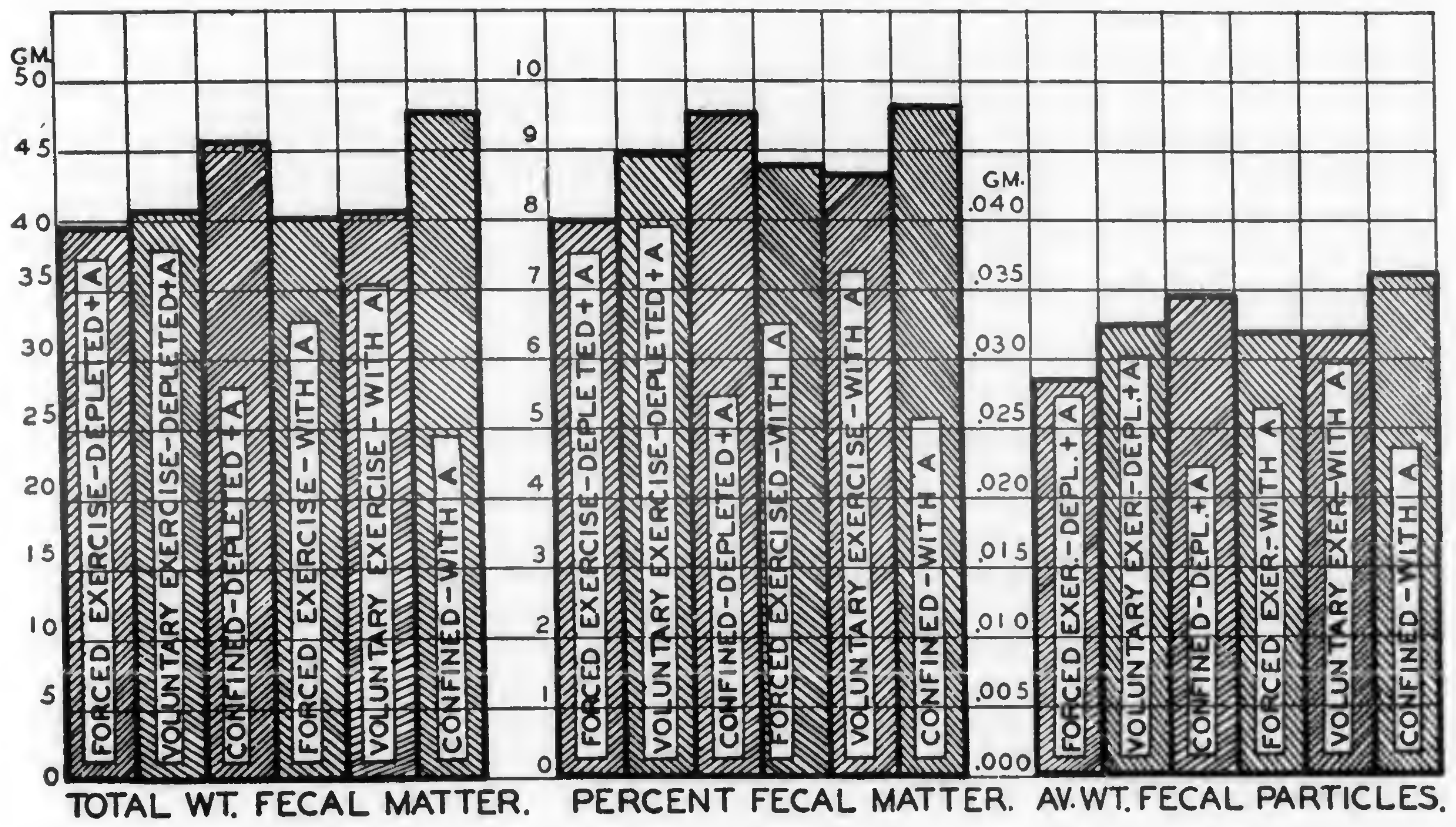

Fig. 3 The effect of exercise on the total weight of fecal matter, percentage of fecal matter and average weight of fecal particles voided by young rats while receiving diets comparable in all respects except as to the vitamin A content.

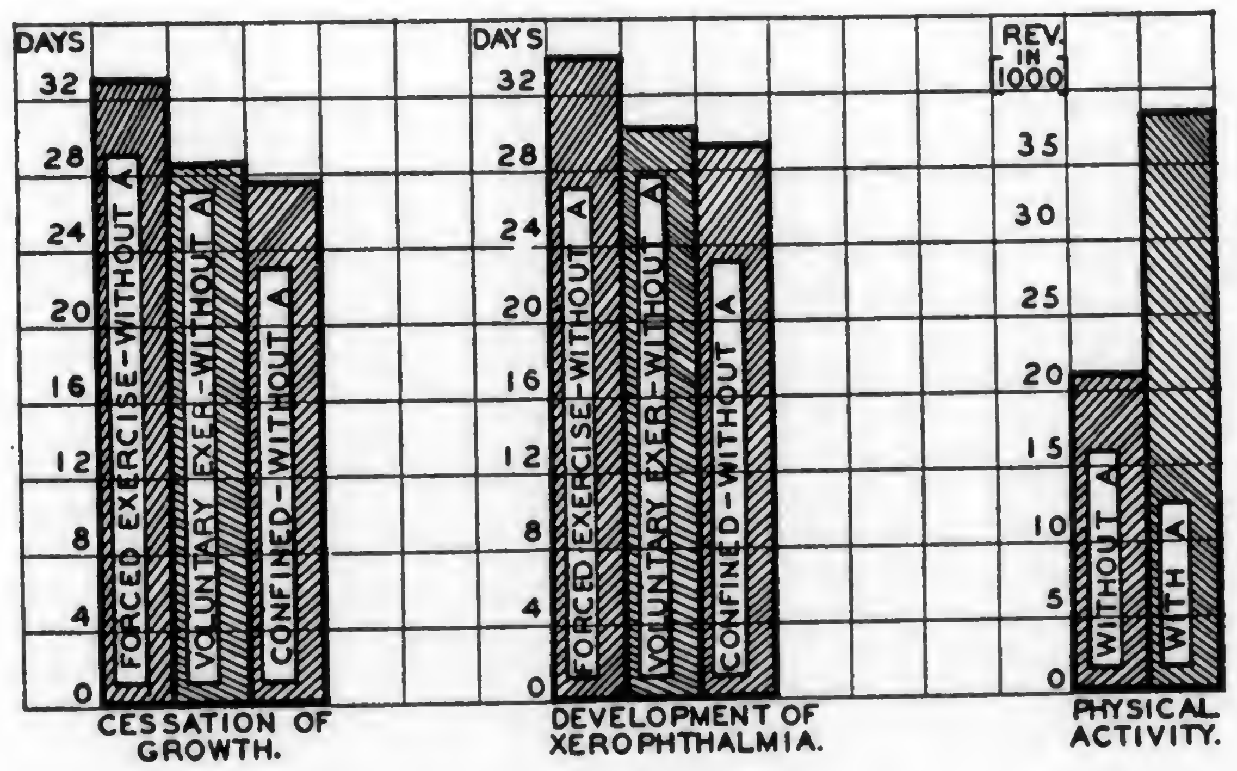

Fig. 4 The effect of exercise on the average time required for the cessation of growth and for the development of the symptoms of xerophthalmia among young rats receiving a diet deficient in vitamin A. This figure also shows the relative activity (as measured by rotation of the cage wheel) of comparable groups of young rats, of which the animals of one group received daily 2 U.S.P. units of vitamin A throughout the experiment, while the animals of the other group received no vitamin A supplement during the first part of the experiment but received 2 U.S.P. units of vitamin A daily during the last 35 days.

an average, 33 days to bring the animals of the forced exercise group to a constant body weight, while similar animals in the confined cages required an average of 27.5 days. Likewise, an average of 34 days was required to produce definite symptoms of xerophthalmia among the animals of the forced exercised group, while those of the confined group required only 29.6 days. These data are not in agreement with those obtained by Keith and Mitchell ('23). However, the two experimental procedures can hardly be considered comparable. It seems possible that the vitamin A requirement of a young animal may be more definitely related to its rate of growth than to its physical activity. It has been frequently observed in this laboratory in connection with our regular vitamin A assays that those animals which make the most rapid rate of growth during the depletion period also are the first to develop the symptoms of vitamin A deficiency. Hence, it appears logical to assume that the confined animals which made the greatest increase in body weight and in consequence developed the greatest demand for the vitamin, should show the earliest symptoms of the deficiency.

Data are also presented in figure 4 to show the effect of vitamin A intake on the physical activity of two comparable groups of animals while being maintained in the freely rotating cages. These data represent the average number of voluntary rotations made by the animals of the two groups. It is to be noted that those animals which had been previously depleted of their vitamin A reserves made a total of 22,000 revolutions during the experimental period, while those animals which received 2 U.S.P. units of vitamin A daily made a total of 38,000 revolutions in a similar period.

It was also observed that a number of the animals composing the voluntary exercise group did not register an appreciable number of revolutions during their first week in the rotating cages. But with increased familiarity with their cage-quarters, all animals of this group showed greater inclination to rotate the wheel. In the case of those animals receiving 2 U.S.P. units of vitamin A daily (fig. 5), this increase in activity continued throughout the experimental period but did not equal the activity previously observed among young animals receiving the breeding colony diet. Those animals which received the vitamin A deficient diet without supplement showed weekly increases in the number of rotations until the end of the fourth week when slight evidences of vitamin A deficiency were becoming apparent. During the following week, these animals showed a marked decrease in activity which continued through the seventh week, although each animal of this group received 2 U.S.P. units of vitamin A daily beginning with the sixth week of the experiment. During the eighth, ninth and tenth week, all animals of this group showed a definite return to activity. In spite of this rapid return to activity the average number of rotations made by this group of animals was always considerably less than the number of rotations made during corresponding weeks of the experiment by the animals in a comparable group which had received the vitamin supplement

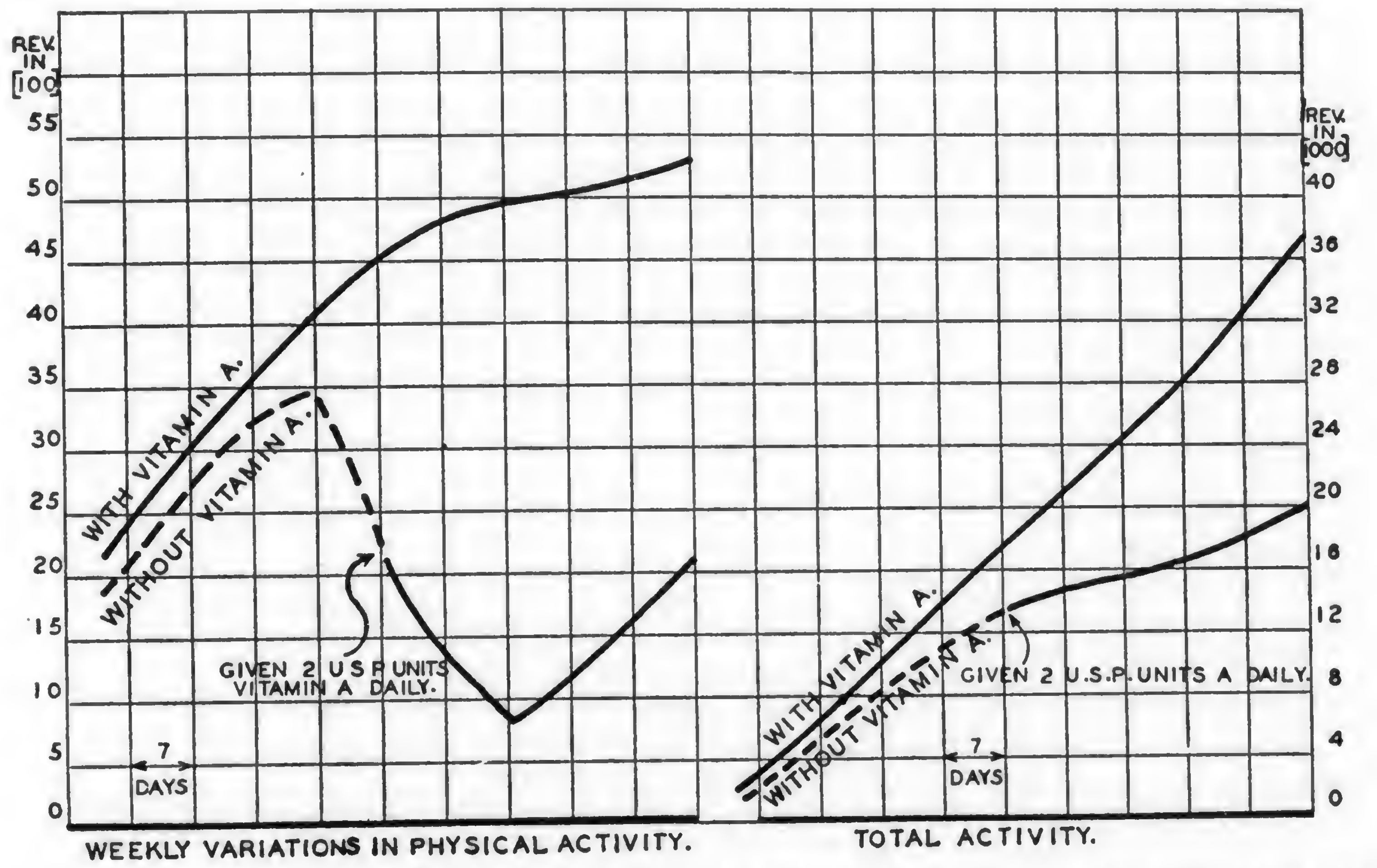

Fig. 5 The average weekly variation in physical activity and also the total activity of two groups of young rats maintained under comparable conditions in all respects except as to their vitamin A intake.

from the beginning of the experiment. These findings further emphasize the importance of an adequate vitamin A intake in the promotion of vigorous physical activity.

SUMMARY

Experiments carried out with carefully selected groups of rats as experimental subjects yielded data to show that under comparable conditions of experimentation less food was consumed, smaller increases in body weight were made and less severe symptoms of vitamin A deficiency developed when the animals were forced to exercise than when allowed to exercise voluntarily or when confined in the usual type of cage.

Although animals maintained under the condition of forced exercise exhibited the greatest efficiency of food utilization, they voided the greatest number of fecal pellets, thus indicating the beneficial effect of exercise on intestinal motility.

Animals maintained under the condition of voluntary exercise and which received daily allotments of vitamin A were more active physically than litter mates which did not receive the vitamin A supplement, further indicating a relationship between completeness of diet and physical activity.

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Dry Skimmilk in Rations For Growing, Laying and Breeding Fowls

Bulletin 381
May, 1939

The Pennsylvania State College
SCHOOL OF AGRICULTURE
AND EXPERIMENT STATION
at State College, Pennsylvania

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PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION

BULLETIN 381[*] STATE COLLEGE, PA. MAY 1939

Dry Skimmilk in Rations for Growing, Laying and Breeding Fowls

WALTHER H. OTT, H. C. KNANDEL, AND R. V. BOUCHER[†]

THE nutritive value of a food is dependent upon its total nutritive energy and upon its content of specific nutritive essentials, such as vitamins, proteins and minerals. Dry skimmilk is recognized as a valuable source of minerals, water-soluble vitamins and proteins of high biological value. Dry skimmilk is widely used in poultry rations because of its beneficial effects on growth, egg production, hatchability and other biological processes.

Although qualitative results have been reported from time to time, there are few records of attempts to determine the most efficient quantity of dry skimmilk to use in the poultry ration. From a practical standpoint, it would be desirable to have information regarding the approximate amount of dry skimmilk to use with meat and fish protein concentrates for best results throughout the growing, laying and breeding periods.

The work herein reported is a study of the effects of dry skimmilk in combination with meat and fish protein concentrates in the ration for growing chicks and laying and breeding fowls. The investigation consisted of three experiments, each of 72 weeks duration, with Single Comb White Leghorns of The Pennsylvania State College strain.

The all-mash basal ration was formulated with the idea that it should meet all known nutritive requirements and that the constituents should conform to best present-day commercial practice in this area, except that it should contain no milk products. Thus, the no-milk basal ration was designed to reveal whether or not the addition of dry skimmilk would improve an already high-grade ration.

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EXPERIMENTAL PROCEDURE WITH GROWING CHICKS

Eight all-mash rations, varying primarily in content of dry skimmilk, meat scraps and fish meal, were prepared when needed and fed as outlined in tables 1 and 2. The content of yellow corn, limestone and steamed bone meal was adjusted to keep the protein, calcium and phosphorus contents uniform, as indicated in these tables. Feed consumption was recorded in pounds at bi-weekly intervals for the first 24 weeks and every 4 weeks thereafter.

Chicks from The Pennsylvania State College breeding flock were used in the first and third years. Progeny of the pullets used in the first year's experiment were used for the second year's experiment. Hatching dates for the three years were: May 16, 1935; May 27, 1936; and May 25, 1937.

Table 1.—Formulae of rations fed from day-old to 12 weeks.

INGREDIENT	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Standard wheat middlings	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Wheat bran	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Ground heavy oats	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Alfalfa leaf meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	.50	.50	.50	.50	.50	.50	.50	.50
Cod liver oil	.50	.50	.50	.50	.50	.50	.50	.50
Ground yellow corn	50.00	49.30	48.59	47.90	47.20	46.50	45.80	45.09
Meat scraps	6.50	6.13	5.77	5.40	5.03	4.66	4.29	3.93
Fish meal	6.50	6.13	5.77	5.40	5.03	4.66	4.29	3.93
Ground limestone	1.00	1.01	1.02	1.03	1.04	1.05	1.07	1.08
Steamed bonemeal		.18	.35	.52	.70	.88	1.05	1.22
Dry skimmilk		1.25	2.50	3.75	5.00	6.25	7.50	8.75
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Mean chemical analyses: protein, 16.9%; calcium, 1.7%; phosphorus, 1%.

Table 2.—Formulae of rations fed from 12 to 72 weeks.

INGREDIENT	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Standard wheat middlings	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Wheat bran	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Ground heavy oats	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Alfalfa leaf meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	.50	.50	.50	.50	.50	.50	.50	.50
Cod liver oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ground yellow corn	50.50	49.80	49.09	48.40	47.70	47.00	46.30	45.59
Meat scraps	4.50	4.13	3.77	3.40	3.03	2.66	2.29	1.93
Fish meal	4.50	4.13	3.77	3.40	3.03	2.66	2.29	1.93
Ground limestone	4.00	4.01	4.02	4.03	4.04	4.05	4.07	4.08
Steamed bonemeal		.18	.35	.52	.70	.88	1.05	1.22
Dry skimmilk		1.25	2.50	3.75	5.00	6.25	7.50	8.75
Grit and oyster shell, ad lib.								
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Mean chemical analyses: protein, 14.4%; calcium, 2.4%; phosphorus, 1.7%.

All chicks were weighed and wing-banded at hatching time. At bi-weekly intervals during the first 24 weeks each year, all chicks were individually weighed in grams and the mean weight per bird for each group was converted to pounds.

Selection, at the time of reduction in number of birds in each group, was based upon body weight and physical development so that the mean body weight of each group was unchanged. Similarly constructed and equipped pens were used and similar management practices were followed with all groups.

In an effort to reduce coccidiosis infestation, molasses was used in the drinking water every tenth day from the twelfth to eighteenth week at the rate of 1 ounce per gallon during the first year and 5 ounces per gallon during the second year. During the third year, equal amounts of a 15 per cent beta-lactose mash were fed each group for two days following each bi-weekly weighing from the tenth to the twentieth week.

In the first year, 1400 day-old chicks were distributed at random to eight pens of a continuous type laying house in which they were kept throughout the experiment. Males were removed at four and six weeks of age and placed in starting batteries for the broiler production phase of the work. At 10 weeks 8 males were selected from each group in the starting batteries and placed with the same group of females from which they had been removed. The number of pullets in each group was reduced to 70 at 10 weeks of age, and at 20 weeks the number was further reduced to 50 females and 5 males per group.

Chicks were obtained for the second year's experiment by incubating 300 eggs from each group of pullets in the first year's study. Chicks were sexed at hatching time, and 85 females from each group were placed in hot-water heated starting batteries in a building constructed for battery brooding. Each of the eight groups of chicks received the same ration as their parents. At eight weeks of age all groups were transferred to pens in a continuous type of brooding and rearing building. Each pen was equipped with a wire-screened sunporch. The number of females was reduced to 55 per group at 21 weeks of age and to 50 per group at the end of the twenty-fourth week. Five males were placed with each group.

For the third year's experiment, chicks obtained from The Pennsylvania State College breeding flock were distributed at random in eight groups so that each group consisted of 100 females and 12 males. The chicks were brooded in the starting batteries and transferred at 10 weeks of age to pens in the continuous type of brooding and rearing building. The total number of chicks in each group was reduced to 100 at the end of the second week, and to 50 females and 6 males at 21 weeks of age.

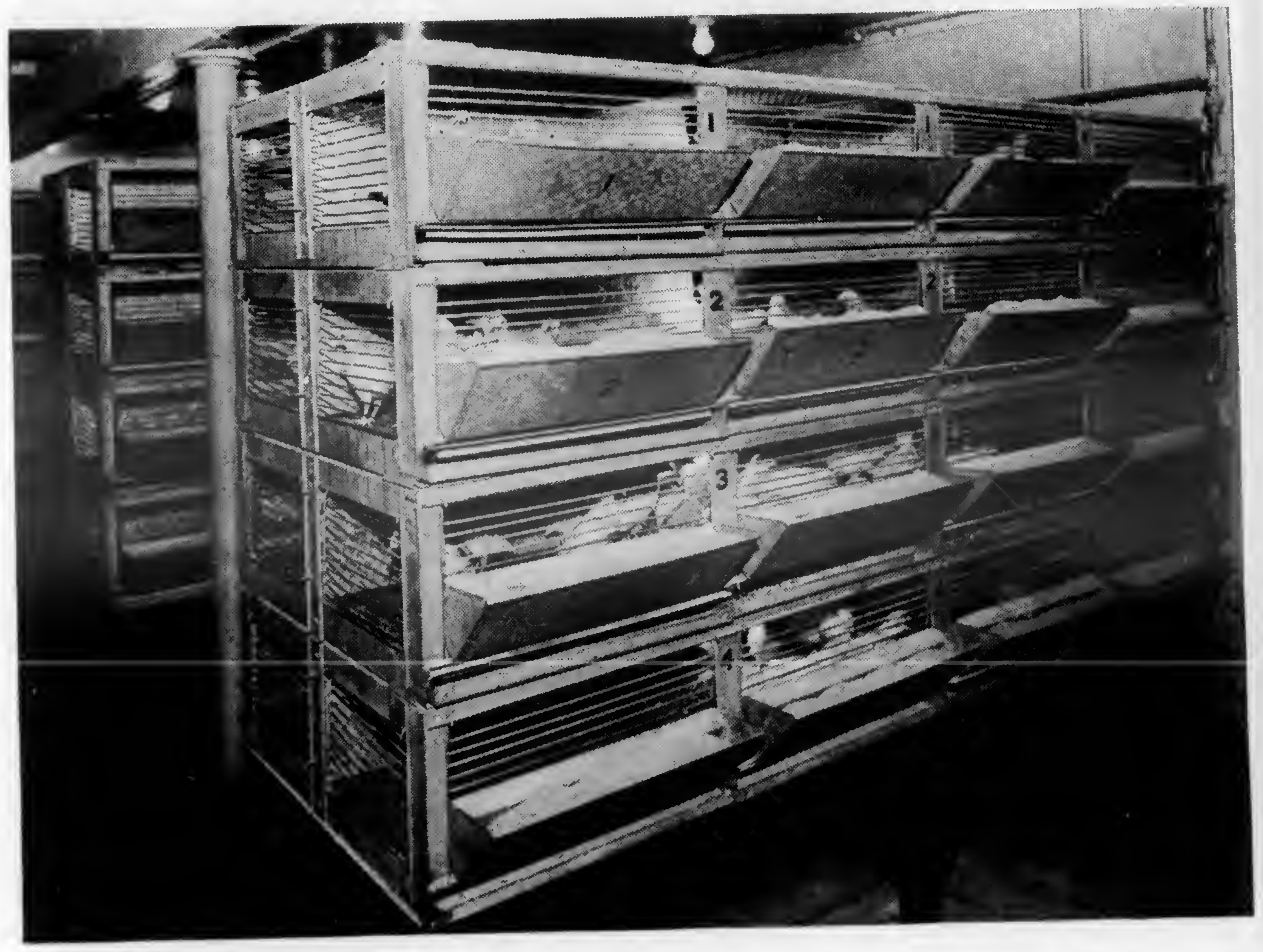

Fig. 1.—These are the starting batteries in which chicks for the second and third years were brooded.

Two trials were conducted to study the shrinkage in the weight of broilers when shipped alive to market. At four and six weeks of age, males from the first year's experiment were transferred to starting batteries, and each group was continued on the same experimental ration. At 10 weeks of age, 30 of the largest males in each group were weighed, shipped alive by railway express under practical marketing conditions to Pittsburgh, Pa., a distance of approximately 150 miles, and again weighed. This work was repeated in a trial involving 432 Barred Plymouth Rock males, hatched Nov. 26, 1935, and grown and marketed in a manner similar to that of the first trial.

All data were analyzed statistically in order to evaluate the results satisfactorily. Minimum significant differences between means were calculated from the analysis of variance as outlined by Snedecor *(6)* and are included with the tabulated data. These values are calculated so that there is only 1 chance in 20 of a difference as great or greater than the 5 per cent value occurring due to chance alone. The application of these values to the experimental results is illustrated by the examples in table 5. All tabulated data are expressed as mean values per bird. These means are the arithmetic averages for the data involved.

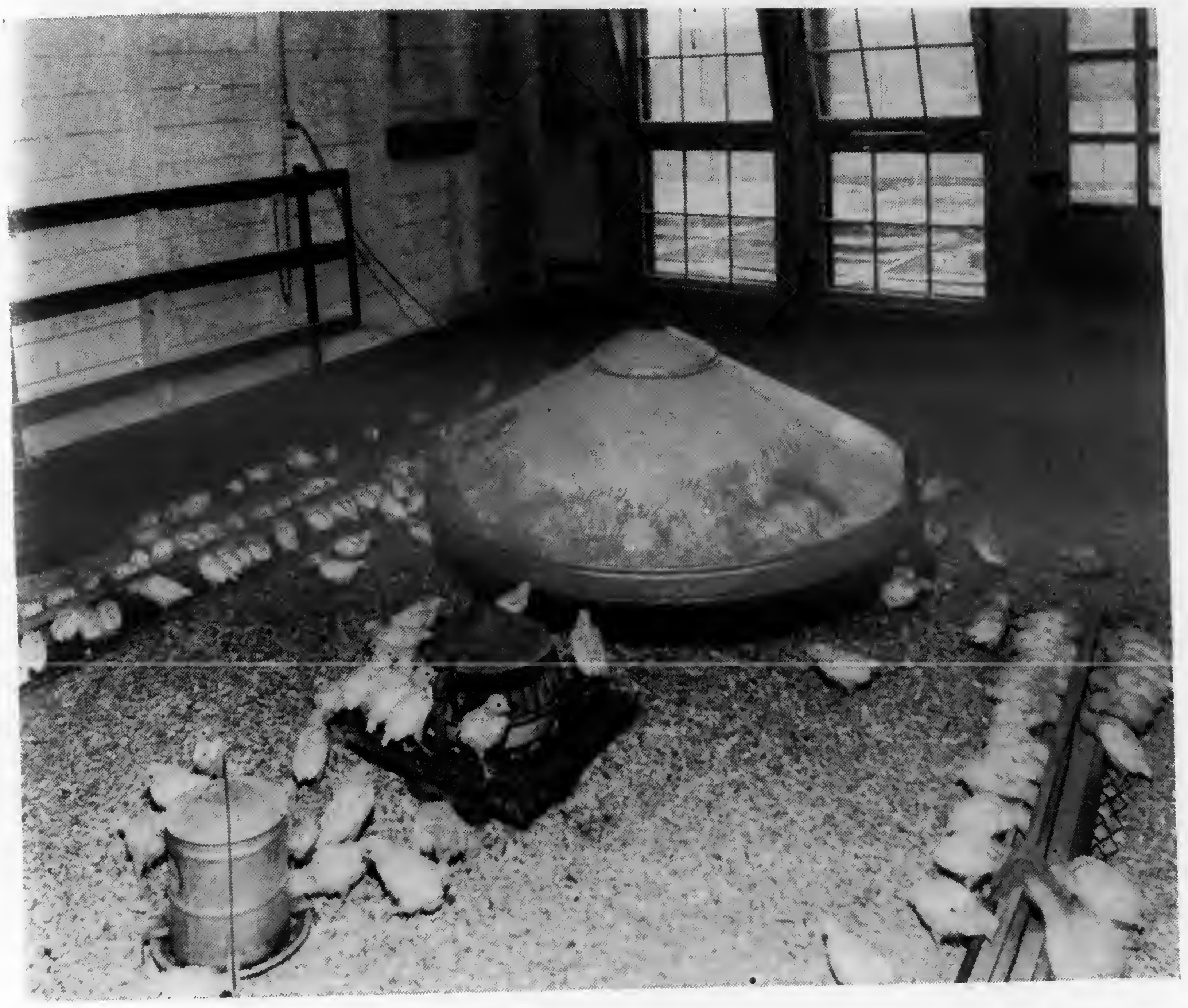

Fig. 2.—This is one of the pens in the continuous type of brooding and rearing building which was used during the latter part of the growing period in the second and third years.

RESULTS WITH GROWING CHICKS

Body weight.—Mean body weight at two weeks of age increased with increasing amounts of dry skimmilk in the ration. At this age the mean weight of each of the eight groups was nearly the same in the first as in the third year. In the second year all groups, especially the groups fed 1.25 and 7.5 per cent dry skimmilk, weighed less than in the first and third years.

At 24 weeks of age, mean body weight definitely increased in all groups from the no-milk group to the 6.25 per cent milk group in the second and third years. In the first year, an increase in weight was evident in all groups from the 1.25 to the 8.75 per cent milk group at 12 weeks of age, but such trends were not marked at 24 weeks.

Up to 24 weeks in the second year, total gains in weight of groups fed 2.5, 3.75, 5 and 7.5 per cent milk were close to the respective gains in the first year. The gains of the no-milk and 1.25 per cent milk groups were considerably lower, and the gains of the 6.25 and 8.75 per cent milk groups were considerably higher, in the second than in the first year. The no-milk group rather consistently made smaller gains up to the twelfth week than did the other groups. During the latter part of the growing period, especially in the first year, the no-milk group on many occasions made greater gains than several of the milk-fed groups.

In order to evaluate more critically the trends in body weight during the growing period, growth constants were calculated for each 2-week interval. The Schmalhausen growth constant, discussed in detail by Lerner and Asmundson *(2)*, was used as a measure of the growth rate. The growth constants in tables 28, 29 and 30 indicate that the rate of growth increased with the amount of dry skimmilk in the ration during the first two weeks in the first and third years and during the first four weeks in the second year. With few exceptions the range in growth rates during each 2-week interval throughout the remainder of the growing period was relatively small.

Feed consumption.—Total feed consumption to 24 weeks of age was closely associated with mean gains in body weight during the same period. Mean feed consumption to 24 weeks in the first and third years increased .028 pounds per bird for each 1.25 per cent milk included in the ration. Group differences in feed consumption were not significant in the first year. Aside from the relatively high values for the 2.5 per cent milk group and the low value for the 5 per cent milk group, feed consumption in the second year increased with the amount of dry skimmilk in the ration. This trend was evident in the third year with the groups fed none to 6.25 per cent milk.

Feed efficiency.—Efficiency of feed utilization increased with the amount of dry skimmilk in the ration during the first six weeks of age in the first and third years, and during the first two weeks of the growing period in the second year, tables 31, 32 and 33. Feed efficiency values of the eight groups were close together throughout the remainder of the growing period.

Another measure of efficiency is the number of pounds of gain in excess of the gain of the no-milk group per pound of dry skimmilk consumed. With the gain of the no-milk group as the basis for calculating excess gains, dry skimmilk efficiency would be constant for the seven milk groups if their excess gains were directly proportional to the respective amounts of dry skimmilk consumed.

According to this measure of dry skimmilk efficiency for extra gains in weight, maximum efficiency occurred in the groups fed 1.25 and 2.5 per cent milk within the first 6, first 8 and first 12 weeks of age in the first, second and third years, respectively. Efficiency in the remaining groups was maximum within the first six, first four and first eight weeks of age in the first, second and third years, respectively. During each of the various phases of the growing period listed in tables 34, 35 and 36, efficiency tended to decrease as the amount of dry skimmilk increased.

It is evident that response to dry skimmilk was greatest during the early part of the growing period. Table 3 was compiled from data of the first and third years to estimate at what time during the growing period the use of milk apparently ceased to be beneficial in this investigation.

Mean gain in body weight per pullet from day-old to 24 weeks increased at the rate of .016 pounds for each 1.25 per cent milk included in the ration. The fact that the mean gain from day-old to 10 weeks of age also increased at the rate of .016 pounds per 1.25 per cent milk indicates that the differences in body weight between groups were already attained at approximately 10 weeks of age. Differences in feed consumption to 10 weeks, however, were about one-third as great as the differences at 24 weeks. Thus, for the period from 10 to 24 weeks feed consumption per bird in-

Table 3.—Regression coefficients of gain in body weight, feed consumption and feed efficiency on per cent dry skimmilk in the first and third years.*

AGE	GAIN IN BODY WEIGHT	TOTAL FEED CONSUMPTION	POUNDS OF GAIN PER POUND OF FEED
	lbs.	*lbs.*	*lbs.*
Day-old to 4 weeks	.004	.005	.0019
4 to 8 weeks	.010	.019	.0020
8 to 12 weeks	.005	.014	.0005
12 to 16 weeks	.004	.014	.0003
16 to 20 weeks	-.005	.004	-.0009
20 to 24 weeks	-.001	.052	-.0010
Day-old to 6 weeks	.010	.007	.0032
Day-old to 8 weeks	.014	.024	.0018
Day-old to 10 weeks	.016	.035	.0013
Day-old to 12 weeks	.019	.038	.0012
Day-old to 24 weeks	.016	.108	0
8 to 24 weeks	.002	.084	-.0004
10 to 24 weeks	0	.073	-.0006

* The values in this table represent the average change in gain, feed intake and feed efficiency for each change of 1.25 per cent dry skimmilk in the ration. For example, the gain in body weight per pullet from day-old to 6 weeks increased .01 pounds per 1.25 per cent increase in dry skimmilk. On this basis the expected gain of the group fed 8.75 per cent milk (seven times 1.25) is .07 pounds per pullet higher than the expected gain of the no-milk group from day-old to 6 weeks. A negative value indicates that the particular variable decreased that amount for each 1.25 per cent increase in dry skimmilk in the ration.

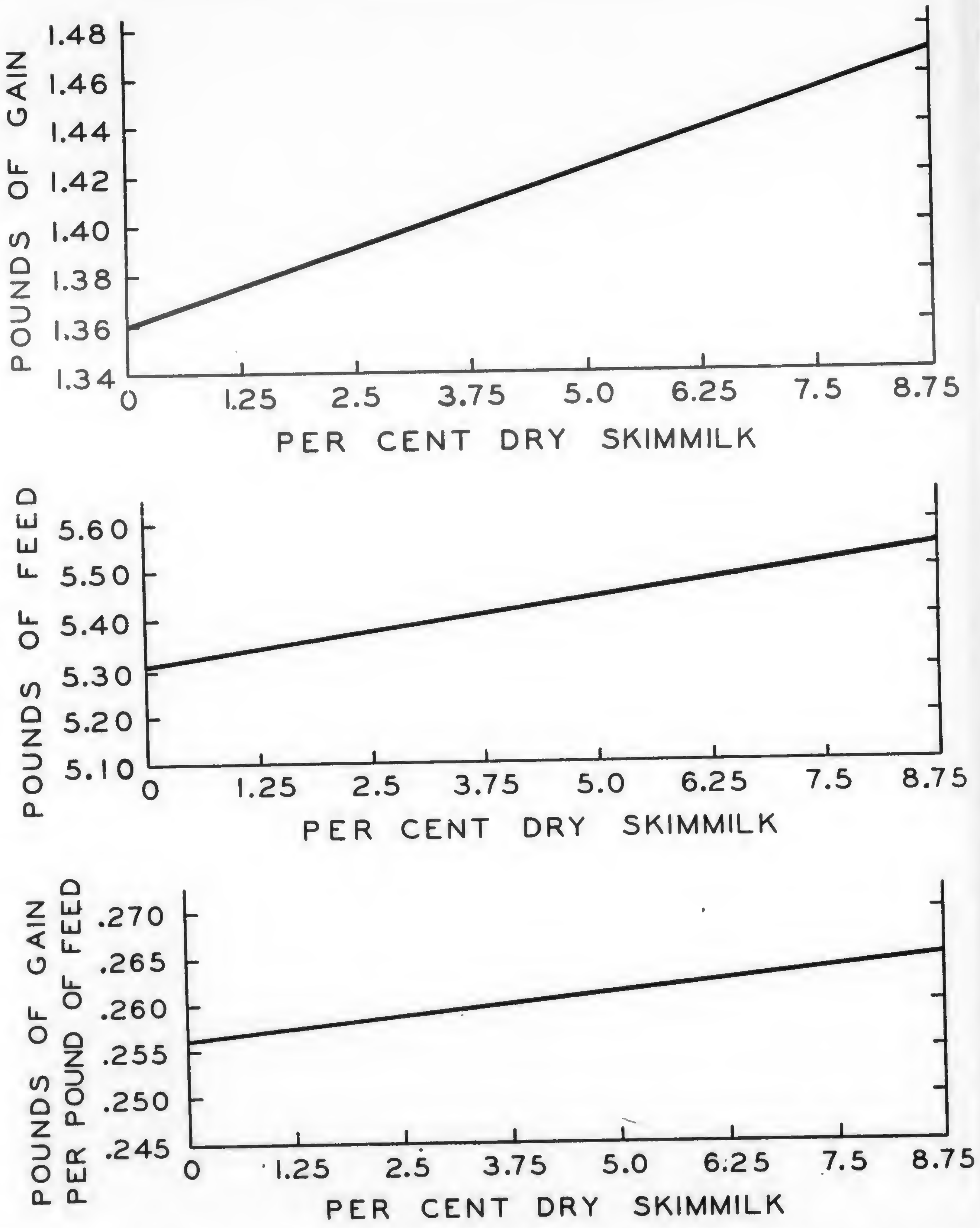

Fig. 3.—During the first 10 weeks of age in the first and third years, gain in weight, feed consumption and feed efficiency increased with increasing amounts of dry skimmilk in all-mash rations.

creased .073 pounds per 1.25 per cent milk in the ration, while gains in weight in the eight groups were of the same magnitude.

Feed efficiency increased with the increase in amount of milk in the ration in the early part of the growing period, but decreased in the later part. The tendency of weight gain, feed intake and feed efficiency to increase with increasing milk levels during the first 10 weeks of growth is illustrated graphically in figure 3. Trends of these three variables from 10 to 24 weeks are shown in figure 4.

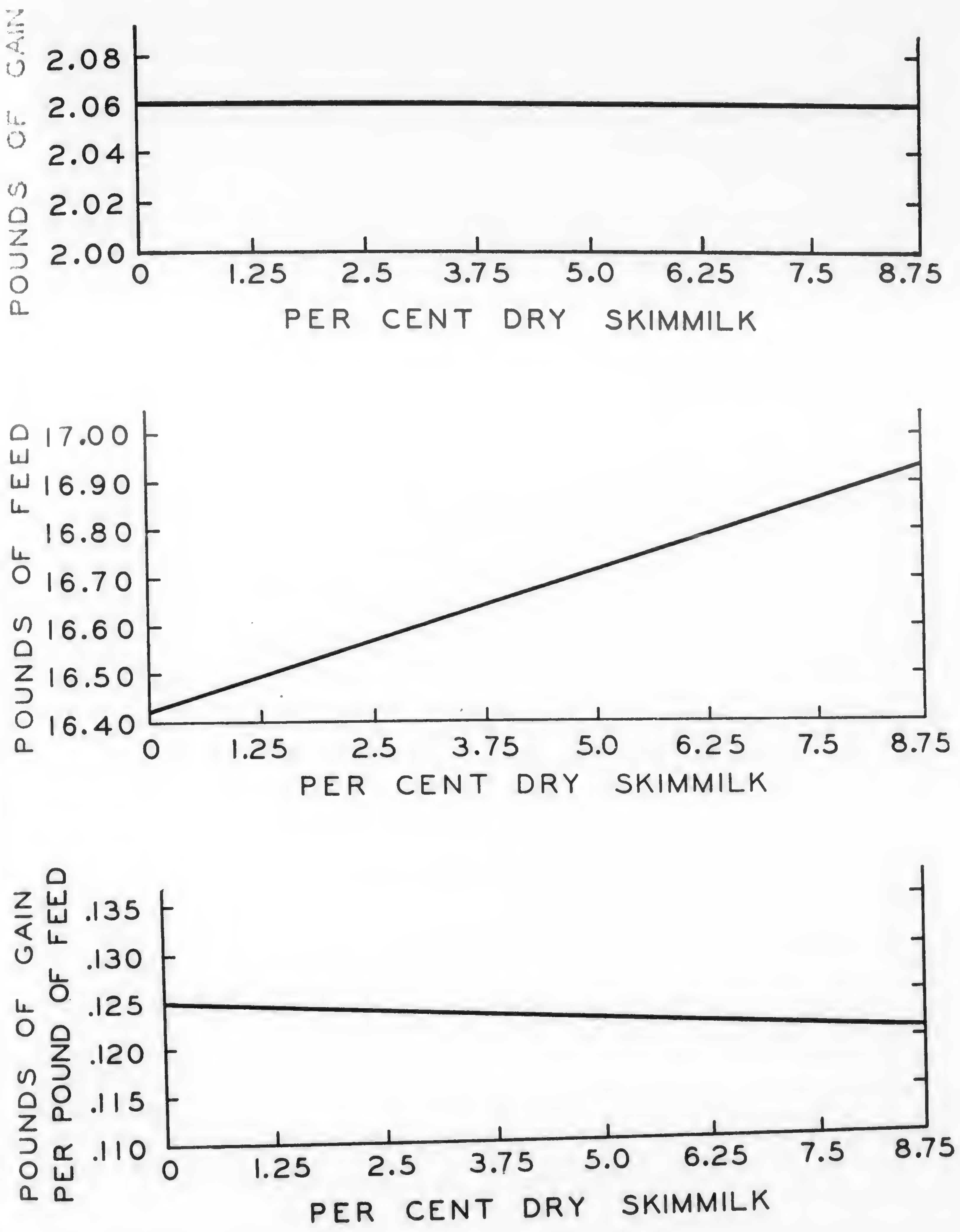

Fig. 4.—From 10 to 24 weeks of age in the first and third years, all groups tended to make equal gains in weight, while feed consumption increased and feed efficiency decreased with increasing amounts of dry skimmilk in all-mash rations.

Mortality.—Differences in total mortality in the growing period were not significant in any of the three years. Although an outbreak of coccidiosis at about the fourteenth week in the second year affected all eight groups, losses from the attack were least in the groups fed 5 to 8.75 per cent milk. The deleterious effects of the infestation were apparent for several weeks in groups fed no milk to 3.75 per cent milk. Losses from any other causative agent were so few in number that no significant trends were evident.

Mortality was considerably lower in the first year than in the second or third. Mortality was higher in the early part of the growing period than in the latter part. Although some birds were removed because of poor health, this procedure appeared to have little bearing on the general results.

Broiler production.—Greatest shrinkage of live weight in transit was in the no-milk group in each of the two experiments on shipping broilers to market. The 7.5 per cent milk group in the first trial and the 8.75 per cent milk group in the second trial also were high in shrinkage. In the first trial, the 8.75 per cent milk group tied with the 2.5 per cent milk group for lowest shrinkage.

SUMMARY OF RESULTS WITH GROWING CHICKS

In view of the uniform increase in dry skimmilk content of the eight rations, the only significant growth responses to be associated with this variable occurred in the early period of growth. Rate of growth was directly related to milk level during the first two weeks. Those groups which grew slowly at first tended to grow relatively more rapidly later in the growing season, indicating the tendency of all groups to reach approximately equal mature body weights.

Total gain in body weight from day-old to 24 weeks increased with increasing amounts of milk in the ration. The magnitude of the increase was accounted for by the tenth week, all eight groups gaining nearly equal amounts in the period from 10 to 24 weeks of age. Feed consumption also increased with increasing amounts of milk, but at 10 weeks the differences were only one-third as great as the differences at 24 weeks. Feed efficiency increased with the increasing per cent of milk during the early part of the growing period, but decreased during the last part of the growing period.

Most efficient extra gains in weight in each group were made prior to the twelfth week of age. The groups fed 1.25 and 2.5 per cent milk were highest in dry skimmilk efficiency, maximum values in these groups occurring from the sixth to twelfth weeks. The higher-milk groups were considerably less efficient for extra gains in weight.

It appears that the maximum beneficial effects of dry skimmilk in the ration occurred prior to the tenth week of age. From the standpoint of rapid early growth and efficient feed utilization, best results were obtained with rations containing 1.25 and 2.5 per cent dry skimmilk. This investigation showed no value for the continued use of dry skimmilk between the tenth and twenty-fourth weeks. However, this does not prove that satisfactory results in the subsequent laying year would be obtained by feeding dry skimmilk only during the early part of the growing period.

Two factors besides per cent of dry skimmilk in the ration apparently had significant effects on various results in this investigation: First, each group in the first and second years represents first and second generations respectively on each of the eight rations. Second, the breeding flock from which the first year's chicks were obtained had been fed a grain and mash ration with 7.25 per cent dry skimmilk in the mash. This ration was thus equivalent in dry skimmilk content to the ration of the group fed 3.75 per cent milk. Gains up to the twenty-fourth week of age were below average in the second generation of birds fed rations containing none and 1.25 per cent dry skimmilk. Gains were above average in the second generation of birds fed rations containing 6.25 and 8.75 per cent dry skimmilk.

Highest shrinkage in weight of broilers shipped alive to market occurred with birds fed no dry skimmilk. Evidently the use of milk in the ration did not increase the per cent of shrinkage.

EXPERIMENTAL PROCEDURE WITH LAYING HENS

All females each year were leg-banded at 21 weeks of age and individually weighed at 4-week intervals from 24 to 72 weeks, inclusive. They were trapped for date of first egg and individual egg production in the first two years, and only for date of first egg in the third year. Eggs produced daily by each group were weighed as a unit.

The individual body weights at sexual maturity given in table 17 were calculated by linear interpolation from the regularly-obtained weights immediately preceding and following the date of first egg. This procedure was first justified by comparison of the calculated values with the actual weights obtained in the first and second years. The mean interpolated weights for the several groups ranged from .2 to 2.5 per cent higher than the observed weights, the average deviation being only 1 per cent. In view of this relatively close agreement, the actual weights at date of first egg were not obtained in the third year.

For the hatchability studies each year, all eggs were collected from each group on successive days until the desired number of eggs was obtained for incubation. Because of low egg production during April of the second year, fewer eggs than the desired number were set in several of the groups. Males were rotated from pen to pen once weekly the first year, and not rotated in the second and third years. Five males in the first and second years and six males in the third year were placed with each group. The males were removed when the hatchability tests were completed.

During the laying period in the first year, the birds remained in the same pens in the laying house in which they had been reared. In the second and third years, the birds were transferred at 21 weeks of age from the brooding and rearing building to the laying house.

In the first year, outdoor runs free of vegetation were available to the birds, except during the period from 34 to 44 weeks of age. A wet mash made by moistening a small amount of each ration with water was fed respectively to each group from the sixty-fourth to the seventy-second week, inclusive. In the second year the same type of wet mash was fed from time to time during the laying period. Wet mash was not fed in the third year. Artificial morning light was supplied each year during the winter months and near the end of the laying period to provide a 14-hour day.

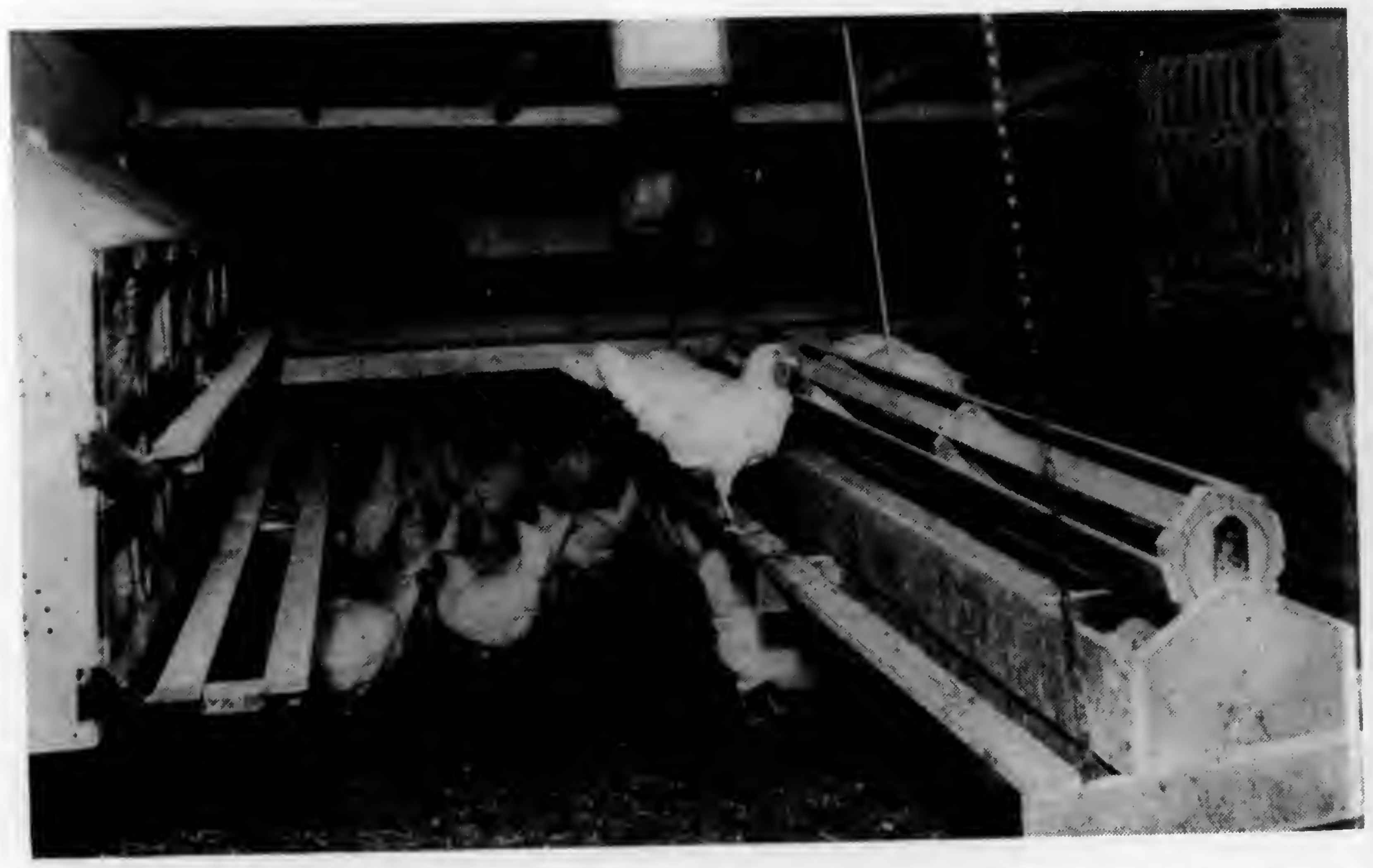

Fig. 5.—This is one of the pens in the continuous type laying house which was used during the entire first year and during the laying period in the second and third years.

Ten eggs selected at random from one day's egg production by each group were used in egg quality studies conducted at various times during the first year. The albumen was scored according to the standards of Van Wagenen and Wilgus *(7)*, and the amount of firm albumen was measured volumetrically as outlined by Lorenz and Almquist *(3)*. Yolk index was determined by the method of Sharp and Powell *(5)*, and yolk color with the color rotor of Heiman and Carver *(1)*.

RESULTS WITH LAYING HENS

Body weight.—Maximum body weight was reached at about 32 weeks of age in the first year, at about 28 weeks of age in the second year, and at about 48 weeks of age in the third year. Mean body weights of the eight groups did not differ greatly throughout the first and third years and in the latter part of the second year. In the early part of the second year, groups fed 3.75, 5, 6.25 and 8.75 per cent dry skimmilk were highest, groups fed none, 1.25 and 2.5 per cent milk were intermediate, and the 7.5 per cent milk group was lowest in body weight.

Mean body weight for the laying period increased fairly uniformly from the 1.25 per cent milk group to the 6.25 per cent milk group in all 3 years. The no-milk group was high the first year, and the 7.5 per cent milk group was low in the first and second years. Groups fed 6.25 and 8.75 per cent milk were consistently high, while groups fed 1.25 and 2.5 per cent milk were consistently low in body weight throughout the laying period each year. Mean body weight during the laying period was closely correlated with mean body weight at 24 weeks of age.

Feed consumption.—Differences were not significant between the total feed consumption of groups fed 3.75 to 7.5 per cent milk in the first year and of groups fed 3.75 to 8.75 per cent milk in the second and third years. In the first year, the 2.5 and 8.75 per cent milk groups were high; in the second year, the none, 1.25 and 2.5 per cent milk groups were low; and in the third year, the no-milk group was high and the 1.25 and 2.5 per cent milk groups were low in total feed consumption.

Egg production.—Age at first egg decreased slightly with increasing levels of dry skimmilk in the ration, while weight at first egg decreased negligibly in the first year and increased slightly in the second and third years. Group differences in age and weight at first egg were statistically significant in the second year only. The hens fed 6.25 per cent milk averaged 13 days less for age and .25 pounds more for weight at first egg than did the hens receiving no milk in the ration.

Highest mean egg production in the 3 years was 186 eggs per pullet for the 5 per cent milk group in the third year. The no-milk and 8.75 per cent milk groups were also high in the third year. Lowest mean egg production was 126 eggs per pullet for the no-milk group in the second year. Group differences in mean per cent egg production were highly significant in each year. Mean egg production of the 1.25 and 7.5 per cent milk groups was consistently low in the first and third years. Highest production was in the 2.5 per cent milk group in the first year and in the 5 per cent milk group in the third year. Egg production in the second year increased from the no-milk group to the 7.5 per cent milk group.

Egg weight.—Waters *(8)* has suggested that measurements made from 44 to 64 weeks of age are a good indication of annual egg weight. In the present investigation, egg weight was no longer increasing rapidly and maximum body weight was reached by 44 weeks of age. Egg production was maximum from the forty-fourth to the sixty-fourth week. Mean egg weights for the 44 to 64 week interval were higher than, but closely followed the trends of, the corresponding mean values for the entire laying year.

Mean egg weight of all groups was highest in the first year and lowest in the third year. Egg weight was highest in groups fed 3.75 and 5 per cent milk. Except for the high egg weights in the 6.25 and 8.75 per cent milk groups, the trend in the second year was similar to that in the first. In the third year, egg weight increased with increasing milk levels except for the very low value for the 5 per cent milk group.

Hatchability.—Per cent hatch of fertile eggs increased with increasing amounts of dry skimmilk in the ration in the first and third years. Hatchability in the second year was very similar to that in the first year aside from the wide deviations of the no-milk and 1.25 per cent milk groups. Differences between group means were highly significant in each year.

Egg quality.—None of the factors of egg quality studied showed significant differences between group means or otherwise indicated any influence of the variables in the ration.

Mortality.—Differences in mortality were not significant in the first or third years but were highly significant in the second year. Total mortality in groups fed 6.25 to 8.75 per cent milk was uniform throughout the 3 years, while mortality was quite variable in the lower milk groups. Leukosis and neurolymphomatosis were the major causes of mortality.

It is apparent from table 37 that the mortality in the second year was considerably lower in the groups fed 6.25 to 8.75 per cent milk than in the lower milk groups. Mortality is summarized in table 4 according to this classification.

Table 4.—Mortality during laying period, 24 to 72 weeks.

AMOUNTS OF MILK IN RATIONS OF GROUPS CLASSED TOGETHER	YEAR First	Second	Third
	Total mortality		
	per cent	*per cent*	*per cent*
None to 5 per cent	29.2	52.4	36.4
6.25 to 8.75 per cent	31.3	28.0	28.0
	Mortality from leukosis and neurolymphomatosis		
None to 5 per cent	12.0	23.6	26.8
6.25 to 8.75 per cent	10.7	7.3	18.7

Mortality was nearly the same in the high-milk and low-milk classes in the first year, but higher in the low-milk class in the third year. Mortality of the progeny (hens in the second year) of hens in the high-milk class was slightly lower than that of their dams (hens in the first year). Mortality of the progeny of hens in the low-milk class was almost two times that of their dams. These same trends are even more evident in the mortality from leukosis and neurolymphomatosis. Although coccidiosis was an important factor in the losses during the growing period of the second year, mortality directly from coccidiosis was negligible in the laying period.

SUMMARY OF RESULTS WITH LAYING HENS

Age at first egg decreased and weight at first egg increased significantly with increasing amounts of dry skimmilk in the ration in the second generation. Egg production of the first generation of pullets on the eight rations was highest on the average in groups fed 2.5 to 5 per cent dry skimmilk. Since egg production of the no-milk and 1.25 per cent milk groups was very low in the second generation, and since egg production of groups fed 5 to 8.75 per cent milk in the first generation did not increase in proportion to the milk level, the most efficient amount of dry skimmilk fed to laying pullets seemed to be from 2.5 to 5 per cent of the all-mash ration.

Rations containing 3.75 per cent milk were most consistent in promoting high egg weight. Hatchability was essentially as satisfactory with 2.5 to 3.75 per cent dry skimmilk as with greater amounts in the ration.

Mortality of first generations on the several rations was practically equal. However, mortality of the second generation was significantly higher on rations containing none to 5 per cent dry skimmilk than on rations with 6.25 to 8.75 per cent milk. This situation was even more pronounced in the mortality from leukosis and neurolymphomatosis.

These observations and the fact that losses from leukosis and neurolymphomatosis were consistently lower in the three high-milk groups suggest a relation between dry skimmilk and the incidence of these particular diseases. In fact, the difference in total mortality between the two classes in the third year is almost entirely due to the difference in losses from the aforenamed diseases. Since the high-milk groups in the second year were not as severely affected with coccidiosis as the low-milk groups, the birds in the low-milk groups may have thus been more susceptible to diseases such as leukosis.

BIOLOGICAL VALUE OF THE RATIONS

The biological value of the proteins in the eight rations was determined by means of a series of tests using 4 rats on each diet, a total of 36 animals being involved. The technique followed was essentially that outlined by Mitchell *(4)*. Whole egg protein was used as the reference standard. Each experimental period was preceded by a 3-day adaptation period during which the test animals received the respective diets under consideration.

Fig. 6.—This set-up was used in determining biological values.

The mean biological values in table 39 indicate that the eight rations did not differ greatly as sources of protein in the test of biological values with rats. At the 9 per cent level, a slight increase in biological values occurred from the 2.5 per cent milk ration to the highest-milk ration. At the 14.5 per cent level, the trend in values from the 2.5 per cent milk ration to the 8.75 per cent milk ration appeared to be curvilinear, the 6.25 per cent milk ration having the highest biological value. In all instances the values for the no-milk and 1.25 per cent milk rations were higher than those for the 2.5 per cent milk ration.

SUMMARY AND CONCLUSIONS

1. Maximum benefits from the use of dry skimmilk in all-mash rations during the growing period were attained during the first 10 weeks of growth.
2. Most efficient gains in body weight on the basis of dry skimmilk intake were made by chicks fed 1.25 and 2.5 per cent dry skimmilk in all-mash rations.
3. From the standpoint of egg production, egg weight, hatchability and dry skimmilk intake, most efficient performance by laying pullets occurred with all-mash rations containing 2.5 to 5 per cent milk. Results were essentially as satisfactory with 2.5 and 3.75 per cent milk as with higher levels.
4. Mortality of the second generation on all-mash rations containing none to 5 per cent dry skimmilk was considerably higher during the laying period than on rations with 6.25 to 8.75 per cent milk.
5. Rate of growth during the first two weeks of age, total feed consumption and gain in weight during the growing period, feed efficiency during the early part of the growing period, body weight at sexual maturity and per cent hatch of fertile eggs increased with increasing percentages of dry skimmilk in the all-mash rations. Age at sexual maturity decreased with increasing amounts of milk in the ration.

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APPENDIX

Table 5.—Mean body weight per pullet, first year.

AGE IN WEEKS	PER CENT DRY SKIMMILK								SIGNIFICANT DIFFERENCES*
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75	
				Growing period					
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old	.08	.08	.08	.08	.08	.08	.08	.08	
2	.22	.22	.23	.23	.23	.24	.25	.24	.01
4	.48	.49	.50	.52	.52	.53	.53	.53	.03
6	.72	.79	.81	.82	.81	.82	.83	.84	.04
8	1.12	1.12	1.14	1.18	1.13	1.20	1.21	1.23	.06
10	1.49	1.44	1.49	1.53	1.47	1.53	1.55	1.58	.07
12	1.83	1.77	1.83	1.89	1.89	1.91	1.90	1.96	.09
14	2.08	2.03	2.05	2.14	2.11	2.13	2.13	2.22	.09
16	2.45	2.40	2.38	2.50	2.47	2.48	2.48	2.59	.09
18	2.70	2.64	2.61	2.72	2.71	2.71	2.68	2.82	.10
20	2.96	2.85	2.88	2.98	2.93	2.93	2.92	3.06	.11
22	3.36	3.20	3.17	3.34	3.19	3.28	3.22	3.38	.14
24	3.68	3.45	3.49	3.60	3.43	3.57	3.51	3.66	.15
				Laying period					
28	3.93	3.77	3.86	3.94	3.89	3.96	3.86	4.04	
32	4.13	4.02	4.10	4.08	4.16	4.25	4.06	4.21	
36	4.00	3.85	3.87	3.91	3.99	4.07	3.90	4.10	.18
40	4.15	3.91	4.02	4.15	4.09	4.15	3.99	4.23	.19
44	4.18	3.91	4.04	4.17	4.00	4.16	3.98	4.17	.20
48	4.26	3.97	3.90	4.21	4.06	4.23	4.09	4.25	.22
52	4.20	3.89	3.89	4.12	4.10	4.19	4.04	4.23	.21
56	4.19	3.96	3.93	4.22	4.05	4.18	3.89	4.19	.23
60	4.16	3.91	3.91	4.15	3.97	4.14	3.95	4.14	.23
64	4.01	3.82	3.78	4.01	3.90	3.98	3.70	4.11	
68	3.96	3.68	3.85	3.87	3.94	3.84	3.74	4.03	
72	4.07	3.67	3.85	3.87	3.82	3.94	3.65	4.01	.27
MEAN 28 to 72	4.10	3.86	3.92	4.06	4.00	4.09	3.90	4.14	

* Minimum differences between means of groups to be significant at a probability of 5 per cent. These values in this and subsequent tables are interpreted according to the following examples: At 24 weeks of age the mean body weight of the 8.75 per cent milk group was 3.66 pounds and of the 5 per cent milk group was 3.43 pounds. The difference of .23 pounds between the two means is significant, since it exceeds the .15 pounds required for significance. However, mean body weights of the 8.75 and 6.25 per cent milk groups are not significantly different, because the difference of .09 pounds is less than the required .15 pounds. Blank spaces indicate that differences in general at those particular ages were not significant.

Table 6.—Mean body weight per pullet, second year.

AGE IN WEEKS	PER CENT DRY SKIMMILK								SIGNIFICANT DIFFERENCES
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75	
				Growing period					
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old	.08	.08	.08	.08	.08	.08	.07	.08	
2	.19	.18	.20	.21	.20	.21	.19	.21	.01
4	.39	.39	.39	.43	.43	.44	.41	.46	.02
6	.75	.75	.79	.79	.82	.84	.77	.87	.03
8	1.08	1.14	1.17	1.13	1.20	1.22	1.11	1.27	.04
10	1.46	1.51	1.59	1.54	1.61	1.65	1.48	1.68	.06
12	1.87	1.93	2.01	1.95	2.00	2.07	1.85	2.08	.06
14	2.19	2.27	2.30	2.27	2.24	2.44	2.18	2.44	.08
16	2.42	2.50	2.58	2.41	2.65	2.72	2.42	2.69	.09
18	2.50	2.64	2.78	2.69	2.79	2.91	2.62	2.90	.10
20	2.78	2.78	3.02	2.96	3.03	3.11	2.84	3.15	.10
22	3.07	3.08	3.20	3.18	3.24	3.34	3.08	3.43	.10
24	3.28	3.32	3.51	3.46	3.55	3.73	3.43	3.89	.13
				Laying period					
28	4.04	4.06	4.14	4.28	4.31	4.38	3.95	4.36	.16
32	4.11	4.09	3.98	4.21	4.13	4.28	3.80	4.22	.18
36	4.00	3.91	4.01	4.06	4.19	4.20	3.74	4.16	.18
40	4.03	3.93	4.08	4.06	4.24	4.32	3.79	4.19	.19
44	3.75	3.74	3.93	4.07	4.20	4.32	3.95	4.29	.23
48	3.97	3.84	3.97	4.01	3.95	4.29	3.87	4.30	.23
52	4.00	3.99	3.98	4.13	4.16	4.31	3.88	4.31	.25
56	3.98	4.04	4.04	4.01	3.96	4.23	3.79	4.25	.24
60	3.91	4.03	3.99	3.88	4.09	4.08	3.58	4.17	.26
64	3.91	3.85	3.91	3.83	4.01	4.03	3.67	4.09	.28
68	3.83	3.66	3.85	3.82	4.01	4.09	3.67	3.97	.29
72	3.93	3.62	3.71	3.95	4.15	3.99	3.75	3.94	.31
MEAN 28 to 72	3.96	3.90	3.97	4.03	4.12	4.21	3.79	4.19	

Table 7.—Mean body weight per pullet, third year.

AGE IN WEEKS	PER CENT DRY SKIMMILK								SIGNIFICANT DIFFERENCES
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75	
				Growing period					
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old	.08	.08	.08	.08	.08	.08	.08	.08	
2	.22	.23	.23	.23	.23	.23	.24	.24	.01
4	.41	.42	.43	.43	.43	.44	.40	.41	.02
6	.74	.75	.79	.80	.79	.83	.78	.78	.03
8	1.06	1.11	1.15	1.13	1.13	1.21	1.14	1.16	.04
10	1.39	1.46	1.52	1.47	1.51	1.60	1.48	1.50	.05
12	1.70	1.80	1.85	1.81	1.83	1.93	1.81	1.83	.05
14	2.09	2.13	2.24	2.21	2.25	2.30	2.21	2.25	.06
16	2.39	2.45	2.49	2.53	2.55	2.61	2.53	2.58	.07
18	2.66	2.68	2.78	2.79	2.80	2.89	2.85	2.84	.08
20	2.88	2.94	3.02	2.95	3.03	3.06	3.03	3.02	.09
22	3.03	3.15	3.31	3.21	3.25	3.24	3.28	3.23	.09
24	3.34	3.54	3.58	3.55	3.59	3.68	3.63	3.58	.12
				Laying period					
28	3.86	3.91	3.85	4.02	3.92	4.07	3.99	3.94	.15
32	3.95	3.95	3.88	3.95	4.01	4.10	4.11	4.07	.16
36	3.81	3.89	3.73	3.90	3.96	4.05	4.02	4.07	.17
40	3.89	3.97	3.74	3.93	3.90	4.16	4.05	4.05	.17
44	3.93	4.00	3.86	4.01	3.95	4.17	4.07	4.06	.17
48	3.96	4.02	3.99	4.11	4.05	4.25	4.18	4.10	.18
52	3.90	3.98	3.96	4.07	4.00	4.15	4.17	4.03	
56	3.92	4.07	3.98	4.06	3.94	4.05	4.28	4.19	.21
60	3.88	3.95	3.94	3.95	4.03	3.95	4.17	4.03	
64	3.86	3.72	3.80	3.83	3.93	3.92	4.13	3.80	.24
68	3.79	3.38	3.87	3.83	3.99	3.88	4.02	3.81	.25
72	3.75	3.53	3.83	3.64	4.03	3.78	3.80	3.83	.21
MEAN 28 to 72	3.88	3.86	3.87	3.94	3.98	4.04	4.08	4.00	

Table 8.—Feed consumption per bird, first year.

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	Growing period							
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2	.52	.51	.53	.55	.53	.54	.53	.55
2 to 4	.94	.94	.94	.96	.95	1.00	.97	1.01
4 to 6	1.22	1.27	1.32	1.32	1.22	1.25	1.23	1.27
6 to 8	1.68	1.47	1.41	1.66	1.66	1.52	1.47	1.76
8 to 10	1.78	1.65	1.68	1.60	1.72	1.65	1.68	1.69
10 to 12	2.03	1.88	1.92	2.05	1.96	1.95	1.92	2.02
12 to 14	1.94	2.01	2.00	1.99	1.86	1.92	1.97	2.10
14 to 16	2.36	2.36	2.23	2.31	2.35	2.33	2.36	2.44
16 to 18	2.35	2.40	2.38	2.36	2.45	2.31	2.39	2.49
18 to 20	2.56	2.60	2.36	2.54	2.50	2.49	2.54	2.44
20 to 22	2.88	2.71	2.70	2.86	2.63	2.71	2.87	3.12
22 to 24	2.91	2.72	2.78	2.83	2.93	2.85	2.96	3.00
Total*	23.17	22.52	22.25	23.03	22.76	22.52	22.89	23.89
	Laying period							
24 to 28	5.95	5.89	5.87	5.82	6.18	5.80	5.90	6.40
28 to 32	7.89	8.04	7.88	7.60	7.90	7.56	7.96	8.03
32 to 36	7.95	8.38	7.77	7.60	7.66	7.98	7.79	7.73
36 to 40	9.00	8.63	8.40	8.32	8.44	8.23	8.80	8.77
40 to 44	8.98	8.74	8.56	8.50	8.25	8.52	8.72	8.63
44 to 48	8.88	8.18	8.27	8.16	7.92	8.22	8.50	8.53
48 to 52	8.32	8.16	8.38	8.17	8.09	8.13	8.57	8.60
52 to 56	7.99	7.78	8.22	8.45	8.09	7.90	7.59	8.14
56 to 60	7.25	7.13	8.05	7.64	7.33	7.17	7.18	7.32
60 to 64	6.03	6.00	7.11	6.70	6.83	6.27	6.14	6.73
64 to 68	5.46	5.51	6.89	5.89	6.43	5.96	6.19	6.54
68 to 72	5.94	5.37	6.69	6.15	6.08	5.63	5.46	6.33
Total†	89.64	87.81	92.09	89.00	89.20	87.37	88.80	91.75

* Differences not significant.
† Minimum difference between totals to be significant: 2.76 lbs.

Table 9.—Feed consumption per bird, second year.

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	Growing period							
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2	.38	.40	.36	.41	.41	.36	.36	.40
2 to 4	.74	.83	.74	.96	.83	.73	.90	.95
4 to 6	1.07	1.19	1.26	1.14	1.18	1.21	1.21	1.31
6 to 8	1.35	1.50	1.75	1.41	1.49	1.53	1.60	1.56
8 to 10	2.24	2.18	2.29	2.08	1.99	2.04	1.96	1.87
10 to 12	2.13	2.07	1.98	1.97	1.93	2.39	2.00	2.08
12 to 14	2.70	2.53	2.93	2.58	2.09	2.77	2.65	2.69
14 to 16	2.40	2.41	2.62	2.30	2.24	2.45	2.44	2.50
16 to 18	2.27	2.37	2.34	2.45	2.38	2.44	2.62	2.56
18 to 20	2.52	2.34	2.77	2.60	2.59	2.57	2.57	2.68
20 to 22	2.62	2.63	2.49	2.53	2.52	2.49	2.41	2.61
22 to 24	2.60	2.74	2.80	2.83	2.85	2.94	2.81	3.21
Total*	23.02	23.19	24.33	23.26	22.50	23.92	23.53	24.42
	Laying period							
24 to 28	6.34	6.60	6.24	6.86	6.71	6.69	6.77	6.66
28 to 32	6.83	7.40	6.57	7.59	6.76	7.23	7.06	7.12
32 to 36	6.55	7.09	6.98	7.30	7.44	7.26	7.04	6.80
36 to 40	7.53	7.71	7.47	7.73	7.99	7.93	7.84	7.21
40 to 44	6.50	7.59	7.21	7.94	8.04	7.66	7.85	7.97
44 to 48	6.45	7.02	7.00	7.28	7.03	7.73	7.75	8.21
48 to 52	6.66	7.22	7.17	7.81	7.39	8.07	7.93	8.88
52 to 56	6.16	7.69	7.07	7.33	6.72	7.32	7.26	7.63
56 to 60	5.68	7.02	6.70	7.11	7.04	6.56	6.83	6.76
60 to 64	5.72	6.25	5.99	6.32	6.70	6.33	6.61	6.28
64 to 68	4.78	5.46	5.47	6.12	6.46	5.95	6.13	5.80
68 to 72	5.44	5.22	5.40	7.03	6.81	6.20	6.45	6.03
Total†	74.64	82.27	79.27	86.42	85.09	84.93	85.52	85.35

* Minimum difference between totals to be significant: 1.28 lbs.
† Minimum difference between totals to be significant: 3.60 lbs.

Table 10.—Feed consumption per bird, third year.

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	Growing period							
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2	.37	.37	.38	.38	.36	.38	.39	.37
2 to 4	.56	.56	.60	.60	.60	.60	.51	.56
4 to 6	1.02	1.04	1.12	1.08	1.06	1.18	1.07	1.12
6 to 8	1.23	1.26	1.32	1.24	1.29	1.44	1.28	1.38
8 to 10	1.34	1.49	1.54	1.40	1.48	1.66	1.49	1.56
10 to 12	1.53	1.44	1.63	1.58	1.55	1.60	1.64	1.51
12 to 14	1.96	1.92	1.96	2.04	2.07	2.02	1.77	1.97
14 to 16	2.12	2.16	2.19	2.32	2.27	2.16	2.26	2.25
16 to 18	2.51	2.49	2.58	2.69	2.52	2.65	2.37	2.60
18 to 20	2.54	2.57	2.68	2.53	2.58	2.58	2.77	2.51
20 to 22	2.28	2.67	2.71	2.90	2.55	2.65	2.83	2.83
22 to 24	2.93	3.00	2.75	2.89	2.95	3.08	3.03	2.82
Total*	20.39	20.97	21.46	21.65	21.28	22.00	21.41	21.48
	Laying period							
24 to 28	6.40	5.98	5.94	6.40	6.24	6.41	6.23	6.00
28 to 32	7.31	6.72	6.73	7.02	7.21	6.96	7.37	7.04
32 to 36	7.02	6.75	6.45	7.04	7.11	7.22	7.19	6.91
36 to 40	7.93	7.48	7.20	7.88	7.73	8.00	8.08	7.59
40 to 44	8.29	7.59	7.73	8.24	7.84	8.43	8.15	8.50
44 to 48	8.39	7.71	7.73	8.22	7.89	8.00	8.31	8.14
48 to 52	7.68	7.30	7.43	7.51	7.43	7.27	7.33	7.43
52 to 56	7.38	7.26	6.95	7.35	7.12	6.71	8.04	7.56
56 to 60	7.30	6.84	6.38	6.65	6.99	6.56	7.03	6.78
60 to 64	7.09	6.19	5.92	6.13	6.48	6.32	6.58	5.75
64 to 68	7.11	5.55	6.40	6.72	7.41	6.22	6.34	6.10
68 to 72	6.29	5.21	5.58	5.38	6.75	5.62	5.04	5.81
Total†	88.19	80.58	80.44	84.54	86.20	83.72	85.69	83.61

* Minimum difference between totals to be significant: .94 lbs.
† Minimum difference between totals to be significant: 3.11 lbs.

Table 11.—Mean per cent egg production, first year.

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
20 to 24	2.6	3.4	2.4	4.6	1.6	2.9	4.1	4.6
24 to 28	28.6	23.8	25.9	30.1	18.2	25.4	30.2	31.6
28 to 32	44.0	36.7	43.9	46.6	42.9	47.4	49.9	47.4
32 to 36	57.5	57.1	59.8	54.5	57.9	62.5	59.1	49.1
36 to 40	54.8	56.2	56.7	46.4	59.2	58.5	58.3	54.5
40 to 44	54.9	57.2	58.9	55.7	61.5	59.2	56.6	59.6
44 to 48	55.2	52.1	64.9	57.1	61.8	60.6	53.7	55.6
48 to 52	55.5	50.4	57.0	57.9	54.7	56.0	60.9	58.5
52 to 56	57.0	51.6	59.1	59.4	60.2	52.1	54.6	59.0
56 to 60	57.1	49.3	61.8	59.3	64.1	55.7	47.5	56.9
60 to 64	34.0	35.1	52.8	52.4	49.1	40.6	39.9	44.0
64 to 68	20.8	27.7	41.2	32.3	38.9	30.2	30.6	33.8
68 to 72	12.6	16.7	29.0	20.2	21.8	17.8	18.8	20.4
Mean*	41.1	39.8	47.2	44.3	45.5	43.8	43.4	44.2

* Minimum difference between means to be significant: 3.3 per cent.

Table 12.—Mean per cent egg production, second year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
20 to 24		.4	.1		.2	2.4	.2	.8
24 to 28	6.2	13.8	18.4	17.6	17.9	25.8	30.0	27.6
28 to 32	52.5	56.2	61.1	65.4	62.0	58.1	65.1	56.3
32 to 36	51.8	58.6	54.0	57.0	56.4	60.3	52.4	47.2
36 to 40	59.1	53.6	59.3	56.1	63.1	55.7	57.0	46.4
40 to 44	40.3	48.5	51.7	55.7	62.3	55.8	52.9	39.1
44 to 48	30.3	35.8	39.9	45.2	49.5	54.2	56.3	50.5
48 to 52	44.0	35.5	48.3	48.5	45.3	54.6	63.3	52.6
52 to 56	45.5	47.0	50.5	45.8	55.6	52.8	57.4	56.9
56 to 60	42.4	45.4	53.1	40.5	53.2	49.4	48.9	51.9
60 to 64	33.2	42.7	51.3	35.0	43.2	46.2	46.1	42.3
64 to 68	24.9	23.8	37.8	32.4	33.5	33.4	38.1	28.0
68 to 72	19.3	10.4	26.4	29.6	32.3	27.7	32.9	13.1
MEAN*	34.6	36.3	42.5	40.7	44.2	44.3	46.2	39.4

* Minimum difference between means to be significant: 4.2 per cent.

Table 13.—Mean per cent egg production, third year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
20 to 24	.4	1.8	3.0	1.4	1.8	1.3	.5	1.1
24 to 28	15.9	24.3	25.4	23.6	27.5	29.0	19.4	24.8
28 to 32	43.5	41.3	47.2	48.6	46.2	43.2	43.5	40.5
32 to 36	65.6	52.3	52.7	60.1	67.5	59.1	61.3	61.0
36 to 40	70.9	60.7	59.5	66.7	70.9	71.9	65.5	72.8
40 to 44	72.9	54.0	61.4	68.7	72.8	70.4	62.5	71.4
44 to 48	74.8	64.0	65.1	65.5	65.4	55.9	57.4	70.4
48 to 52	68.3	64.5	64.6	62.6	64.7	59.0	57.6	64.7
52 to 56	59.7	61.3	59.4	63.5	53.7	60.2	56.5	66.0
56 to 60	62.3	67.2	55.3	59.9	57.3	55.3	55.8	65.2
60 to 64	57.8	54.6	44.2	56.1	55.5	50.7	44.9	44.8
64 to 68	42.6	31.7	35.8	41.6	49.2	37.2	33.6	39.9
68 to 72	24.1	2.8	15.7	12.8	33.7	17.6	12.0	19.2
MEAN*	50.7	44.7	45.3	48.5	51.2	47.0	43.9	49.4

* Minimum difference between means to be significant: 3.8 per cent.

Table 14.—Mean weight per egg, first year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
24 to 28	45.6	44.2	44.8	45.2	46.3	45.5	45.7	45.3
28 to 32	48.8	48.7	48.4	49.4	49.3	49.5	49.6	49.1
32 to 36	51.4	51.4	51.8	52.4	52.7	52.1	51.8	51.6
36 to 40	53.2	53.3	54.0	53.7	55.1	53.2	52.7	53.6
40 to 44	53.6	54.1	54.4	55.8	54.8	53.7	53.7	54.7
44 to 48	54.3	54.7	55.5	56.4	55.5	54.9	54.3	54.9
48 to 52	55.0	54.8	55.4	57.1	56.7	55.3	55.0	55.6
52 to 56	55.4	56.1	55.5	57.5	57.0	56.0	54.5	56.0
56 to 60	55.0	56.8	56.1	57.1	57.5	55.9	55.5	56.6
60 to 64	56.1	56.0	55.3	56.7	56.8	55.3	54.8	55.0
64 to 68	56.7	57.0	56.1	57.7	58.6	57.2	55.7	56.5
68 to 72	56.0	54.5	56.8	57.6	57.8	54.2	56.3	55.7
MEAN 24 to 72	53.4	53.5	53.7	54.7	54.8	53.6	53.3	53.7
MEAN* 44 to 64	55.2	55.7	55.6	57.0	56.7	55.5	54.8	55.6

* Minimum difference between means to be significant: .5 grams.

Table 15.—Mean weight per egg, second year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
24 to 28	43.5	45.4	45.9	46.9	46.7	47.6	46.0	47.3
28 to 32	48.8	49.2	49.9	50.3	49.4	51.0	48.4	50.4
32 to 36	49.2	50.3	50.7	51.2	50.7	52.1	49.0	51.5
36 to 40	51.1	49.7	52.8	52.3	53.2	53.2	50.3	53.3
40 to 44	51.9	51.1	53.8	54.3	53.7	55.1	52.0	54.6
44 to 48	52.6	52.6	54.9	54.9	54.2	56.0	52.5	56.6
48 to 52	53.7	53.6	55.5	55.5	55.2	57.5	53.3	57.6
52 to 56	53.4	52.8	55.0	55.6	55.2	55.9	52.4	56.7
56 to 60	52.8	52.1	54.6	54.2	54.3	53.8	52.2	56.3
60 to 64	53.6	52.6	54.7	54.4	55.0	54.9	52.7	56.6
64 to 68	55.0	53.7	54.4	55.6	56.3	55.3	52.8	59.0
68 to 72	57.6	59.7	57.0	58.0	58.8	57.7	55.6	62.4
MEAN 24 to 72	51.9	51.9	53.3	53.6	53.6	54.2	51.4	55.2
MEAN* 44 to 64	53.2	52.7	54.9	54.9	54.8	55.6	52.6	56.8

* Minimum difference between means to be significant: .5 grams.

Table 16.—Mean weight per egg, third year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
24 to 28	44.6	44.4	44.2	44.8	44.7	45.0	44.2	45.0
28 to 32	48.6	48.4	47.9	48.4	48.6	49.6	48.8	49.0
32 to 36	49.9	49.7	49.2	50.4	50.8	51.0	51.8	51.2
36 to 40	51.6	51.4	50.6	52.0	51.2	53.1	52.5	52.4
40 to 44	52.2	53.0	51.8	53.3	52.2	54.2	53.5	53.3
44 to 48	53.2	53.6	53.0	54.3	52.4	54.1	54.4	54.3
48 to 52	53.6	54.0	53.5	55.1	53.2	55.1	54.6	54.8
52 to 56	54.3	55.0	54.4	55.5	54.2	55.1	55.2	55.2
56 to 60	53.9	54.3	54.6	54.4	53.6	54.6	54.6	55.2
60 to 64	53.6	52.9	54.2	53.9	53.0	54.1	54.0	54.6
64 to 68	54.5	54.2	55.6	54.5	54.0	55.7	55.3	55.2
68 to 72	58.5	58.6	58.3	56.8	57.5	58.6	57.6	58.4
MEAN 24 to 72	52.4	52.5	52.3	52.8	52.1	53.4	53.0	53.2
MEAN* 44 to 64	53.7	53.8	53.9	54.6	53.3	54.6	54.6	54.8

* Minimum difference between means to be significant: .4 grams.

Table 17.—Mean age and body weight at first egg.

DRY SKIMMILK	FIRST YEAR		SECOND YEAR*		THIRD YEAR	
Per cent	*days*	*lbs.*	*days*	*lbs.*	*days*	*lbs.*
None	194.6	3.99	202.3	4.10	200.5	3.91
1.25	198.0	3.82	196.5	3.99	195.8	3.90
2.50	193.5	3.86	192.8	4.06	188.9	3.89
3.75	191.4	3.87	194.8	4.15	193.9	3.96
5.00	198.3	3.95	192.5	4.16	192.0	3.94
6.25	192.1	3.98	188.8	4.25	192.4	4.05
7.50	187.0	3.78	186.9	3.82	195.4	4.05
8.75	188.1	3.94	189.4	4.28	196.8	4.01

* Minimum difference between means to be significant: 5:2 days; .20 lbs.

Table 18.—Hatching results, first year.

DATE HATCHED	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	Number of eggs set							
Feb. 10	165	165	165	165	165	165	165	165
Feb. 24	143	145	150	109	150	139	150	144
March 2	125	115	125	86	140	121	116	130
March 24	131	125	126	126	137	143	130	140
May 26	298	289	300	300	295	301	300	298
July 20	100	100	100	100	100	100	100	100
TOTAL	962	939	966	886	987	969	961	977
	Per cent hatch of fertile eggs							
Feb. 10	63.9	70.7	77.4	69.8	78.5	78.7	82.5	84.5
Feb. 24	55.9	65.6	74.3	49.5	63.6	63.0	69.3	74.4
March 2	64.0	71.7	83.7	66.2	77.4	89.2	79.0	78.4
March 24	72.1	84.8	80.6	75.7	81.9	83.1	80.4	82.1
May 26	76.0	78.3	82.1	79.2	82.8	84.5	80.1	79.2
July 20	59.8	66.7	76.2	64.0	64.5	76.7	65.9	69.5
MEAN*	65.3	73.0	79.0	67.4	74.8	79.2	76.2	78.0

* Minimum difference between means to be significant: 5.4 per cent.

Table 19.—Hatching results, second year.

DATE HATCHED	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	Number of eggs set							
March 22	100	100	100	100	100	100	100	100
March 31	100	100	100	100	100	100	100	100
April 12	150	150	150	150	150	150	150	150
May 3	94	165	165	165	165	165	165	165
May 10	66	55	66	88	76	98	109	99
May 17	69	71	103	107	101	125	148	127
TOTAL	579	641	684	710	692	738	772	741
	Per cent hatch of fertile eggs							
March 22	77.9	37.9	77.6	55.3	65.9	82.2	48.2	57.0
March 31	54.3	44.7	87.9	58.4	41.0	72.4	47.0	60.9
April 12	79.5	55.9	87.1	70.8	79.1	78.1	62.3	61.7
May 3	85.4	73.6	75.3	63.2	81.1	76.2	77.2	75.4
May 10	81.2	79.2	85.2	69.7	93.5	75.7	80.4	88.2
May 17	79.7	76.6	96.0	66.7	87.0	87.6	90.0	79.4
MEAN*	76.3	61.3	84.8	64.0	74.6	78.7	67.5	70.4

* Minimum difference between means to be significant: 11.8 per cent.

Table 20.—Hatching results, third year.

DATE HATCHED	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	Number of eggs set							
Feb. 23	100	100	100	100	100	100	100	100
April 20	100	100	100	100	100	100	100	100
May 31	100	100	100	100	100	100	100	100
TOTAL	300	300	300	300	300	300	300	300
	Per cent hatch of fertile eggs							
Feb. 23	72.6	76.4	65.6	80.9	76.1	86.2	84.6	79.6
April 20	79.3	82.2	75.9	93.3	86.0	81.8	83.3	86.8
May 31	84.6	79.1	75.0	87.6	81.2	88.5	84.4	82.1
MEAN*	78.8	79.2	72.2	87.3	81.1	85.5	84.1	82.8

* Minimum difference between means to be significant: 7.9 per cent.

Table 21.—Mean per cent egg shell of total weight of egg, first year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
28	10.0	9.1	9.8	9.6	9.8	9.7	9.8	9.9
35	9.6	9.7	9.0	9.3	9.4	9.2	9.1	8.9
38	9.6	9.3	9.1	9.5	9.1	9.2	9.0	9.0
43	8.7	8.8	9.1	8.8	9.2	8.2	8.9	8.5
48	9.4	8.4	8.5	9.1	8.7	8.7	8.3	8.4
51	9.2	8.6	9.1	8.3	9.1	8.6	8.4	8.0
57	8.9	8.6	8.6	8.5	8.5	8.0	8.3	8.6
61	8.0	7.7	10.0	9.4	9.2	8.2	8.8	8.2
66	8.1	7.8	6.6	7.2	6.9	7.9	7.1	7.6
MEAN*	9.1	8.7	8.9	8.9	8.9	8.6	8.6	8.6

* Differences not significant.

Table 22.—Mean per cent ash in egg shell, first year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
28	53.9	53.6	53.8	53.6	53.8	53.4	53.8	53.6
35	53.7	53.7	53.5	53.7	53.6	53.4	53.4	53.6
38	53.5	53.5	53.5	53.6	53.5	53.3	53.2	53.5
43	53.4	53.2	53.4	53.3	53.3	53.2	53.5	53.4
48	52.3	52.0	51.9	52.4	52.8	52.9	52.9	53.0
51	51.8	53.4	52.0	52.9	53.2	53.4	53.0	53.0
57	51.5	52.8	51.8	52.9	52.5	52.7	53.0	53.0
61	52.8	53.2	52.2	51.8	52.5	52.7	52.5	52.1
66	52.7	52.6	51.9	52.2	52.5	52.5	52.0	52.6
MEAN*	52.8	53.1	52.7	52.9	53.1	53.1	53.0	53.1

* Differences not significant.

Table 23.—Mean per cent yolk of total weight of egg, first year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
35	29.0	29.2	31.0	30.1	31.5	28.4	29.1	30.0
38	28.6	28.0	27.8	28.1	27.4	27.1	27.7	29.4
43	26.3	27.8	28.2	27.6	28.6	26.9	27.7	28.4
48	29.6	27.8	27.0	28.6	29.5	29.5	28.5	28.3
51	30.1	30.2	29.0	28.3	30.3	29.8	28.9	29.3
57	28.6	28.3	28.9	28.4	29.0	29.3	30.2	29.7
61	30.8	25.8	29.8	30.0	30.7	29.1	29.9	29.1
66	29.9	28.9	30.3	30.2	30.0	29.4	29.1	28.8
MEAN*	29.1	28.2	29.0	28.9	29.6	28.7	28.9	29.1

* Differences not significant.

Table 24.—Mean per cent firm albumen of total albumen, first year.

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
35	56.2	58.6	60.3	57.5	56.4	59.4	58.5	56.3
38	58.9	56.1	56.0	57.7	55.3	57.7	58.3	55.0
43	58.6	55.8	59.3	53.7	58.2	56.6	55.9	57.0
48	54.3	57.0	58.4	50.5	54.8	55.1	60.8	56.0
51	55.7	53.6	51.5	55.0	53.6	51.6	56.8	54.0
57	55.8	55.4	59.1	53.1	56.2	57.3	54.9	55.7
61	54.7	52.2	52.2	54.0	49.1	54.6	55.1	51.8
66	51.4	55.5	53.0	54.9	54.7	52.8	54.3	53.2
MEAN*	55.7	55.5	56.2	54.6	54.8	55.6	56.8	54.9

* Differences not significant.

Table 25.—Mean albumen score, first year.*

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
35	2.05	1.55	1.50	1.90	1.50	1.65	1.85	1.50
38	1.85	1.70	1.80	1.70	1.85	1.85	2.60	2.15
43	1.65	1.75	1.55	1.70	1.55	1.85	2.00	1.50
48	1.85	1.50	1.70	1.70	1.75	1.85	1.70	1.70
51	1.80	1.50	2.00	1.75	1.90	1.75	1.80	1.85
57	1.80	1.55	1.70	1.85	1.70	1.95	1.95	1.75
61	1.75	2.20	1.80	1.95	1.75	1.80	1.90	1.90
66	1.90	1.70	2.20	1.75	1.70	2.00	1.80	1.65
Mean†	1.83	1.68	1.78	1.79	1.71	1.84	1.95	1.75

* Albumen score is a measure of the height and width of the albumen in the broken-out egg.

† Differences not significant.

Table 26.—Mean yolk color, first year.*

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
35	13.7	13.6	13.2	13.7	13.6	13.8	13.8	13.3
38	14.0	14.4	14.4	14.3	14.4	14.2	13.9	13.3
43	13.6	13.8	13.6	13.4	13.6	13.9	13.8	13.3
48	13.6	13.4	12.7	13.8	13.6	13.4	13.1	13.4
51	12.8	12.5	12.9	13.3	13.0	13.2	13.0	13.2
57	12.8	13.2	12.8	12.5	12.7	12.8	12.3	13.2
61	12.0	12.0	12.1	11.3	12.0	12.0	11.7	12.0
66	11.4	11.3	12.1	12.6	12.4	11.8	11.9	11.7
Mean†	13.0	13.0	13.0	13.1	13.2	13.1	12.9	12.9

* A low number indicates a pale yolk.

† Differences not significant.

Table 27.—Mean yolk index, first year.*

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
35	.405	.412	.429	.428	.403	.424	.437	.419
38	.436	.421	.420	.440	.438	.424	.428	.410
43	.489	.433	.458	.463	.458	.411	.457	.435
48	.477	.431	.450	.460	.453	.458	.459	.483
51	.494	.498	.494	.503	.497	.475	.459	.463
57	.485	.471	.458	.463	.442	.412	.452	.472
61	.441	.441	.444	.466	.461	.448	.456	.442
66	.435	.437	.448	.443	.442	.427	.451	.427
Mean†	.457	.443	.450	.458	.449	.435	.449	.443

* Yolk index is the ratio between the height and width of the yolk after the albumen has been removed.

† Differences not significant.

Table 28.—Constants for the rate of growth, first year.*

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
Day-old to 2	1.830	1.860	1.974	1.971	1.952	2.078	2.148	2.118
2 to 4	2.414	2.387	2.310	2.415	2.455	2.405	2.305	2.365
4 to 6	1.602	1.908	1.920	1.828	1.812	1.716	1.743	1.817
6 to 8	2.184	1.767	1.740	1.792	1.653	1.867	1.872	1.897
8 to 10	1.498	1.504	1.576	1.592	1.580	1.477	1.496	1.508
10 to 12	1.665	1.443	1.324	1.471	1.732	1.534	1.418	1.491
12 to 14	1.002	1.075	1.025	.980	.898	.864	.936	1.002
14 to 16	1.508	1.500	1.340	1.396	1.404	1.393	1.354	1.354
16 to 18	.959	.957	.957	.855	.929	.851	.795	.863
18 to 20	.996	.852	1.068	1.006	.859	.876	.943	.897
20 to 22	1.533	1.410	1.164	1.356	1.030	1.340	1.158	1.194
22 to 24	1.177	.971	1.218	.988	.932	1.096	1.113	1.030

* Computed by means of the equation: constant $= \dfrac{\log W_2 - \log W_1}{\log t_2 - \log t_1}$

High values indicate rapid rates of growth.

Table 29.—Constants for the rate of growth, second year.

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
Day-old to 2	1.800	1.740	1.832	1.906	1.848	1.907	1.880	2.016
2 to 4	2.075	2.224	2.044	2.206	2.282	2.241	2.240	2.325
4 to 6	2.633	2.646	2.772	2.380	2.572	2.576	2.475	2.506
6 to 8	1.836	2.058	1.968	1.793	1.912	1.886	1.846	1.900
8 to 10	1.804	1.696	1.853	1.838	1.733	1.813	1.733	1.660
10 to 12	1.672	1.690	1.633	1.654	1.526	1.554	1.548	1.490
12 to 14	1.342	1.318	1.080	1.213	.906	1.310	1.318	1.274
14 to 16	.903	.901	1.024	.558	1.520	.993	.950	.950
16 to 18	.320	.585	.765	1.107	.503	.675	.815	.748
18 to 20	1.176	.563	.894	1.044	.917	.725	.862	.904
20 to 22	1.158	1.210	.674	.862	.793	.845	.976	1.014
22 to 24	.866	.956	1.212	1.074	1.207	1.440	1.414	1.642

Table 30.—Constants for the rate of growth, third year.

Age in Weeks	Per Cent Dry Skimmilk							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
Day-old to 2	1.891	1.994	2.000	2.000	2.007	2.014	2.058	2.042
2 to 4	1.848	1.742	1.810	1.824	1.900	1.887	1.564	1.600
4 to 6	2.386	2.360	2.460	2.474	2.354	2.502	2.680	2.583
6 to 8	1.783	1.913	1.898	1.730	1.810	1.883	1.854	1.954
8 to 10	1.652	1.657	1.641	1.588	1.746	1.696	1.578	1.570
10 to 12	1.408	1.467	1.377	1.451	1.333	1.304	1.410	1.398
12 to 14	1.630	1.328	1.511	1.593	1.662	1.409	1.600	1.650
14 to 16	1.223	1.267	.947	1.248	1.071	1.134	1.223	1.207
16 to 18	.830	.913	1.090	.955	.993	1.012	1.175	.961
18 to 20	1.118	1.025	.912	.633	.851	.628	.702	.686
20 to 22	.635	.806	1.096	.997	.866	.705	.948	.779
22 to 24	1.238	1.517	1.030	1.320	1.277	1.630	1.298	1.360

Table 31.—Efficiency of feed utilization, first year.*

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2	.252	.263	.276	.265	.270	.289	.309	.289
2 to 4	.286	.284	.286	.301	.306	.296	.295	.292
4 to 6	.197	.238	.232	.229	.242	.233	.238	.243
6 to 8	.237	.228	.241	.214	.193	.246	.258	.222
8 to 10	.180	.194	.205	.223	.198	.204	.204	.210
10 to 12	.191	.176	.162	.176	.211	.193	.181	.187
12 to 14	.126	.127	.123	.124	.121	.113	.120	.125
14 to 16	.160	.156	.154	.155	.152	.152	.147	.148
16 to 18	.105	.100	.100	.095	.098	.097	.086	.093
18 to 20	.100	.081	.113	.102	.088	.090	.094	.098
20 to 22	.141	.131	.109	.125	.100	.128	.103	.102
22 to 24	.110	.109	.112	.094	.081	.101	.098	.093

* Pounds of gain per pound of feed consumed.

Table 32.—Efficiency of feed utilization, second year.*

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2	.305	.270	.334	.315	.298	.356	.334	.340
2 to 4	.262	.247	.264	.237	.277	.318	.241	.265
4 to 6	.338	.307	.313	.311	.330	.330	.293	.310
6 to 8	.246	.257	.218	.242	.258	.254	.215	.259
8 to 10	.169	.171	.185	.195	.203	.212	.190	.218
10 to 12	.185	.200	.212	.208	.204	.173	.184	.192
12 to 14	.126	.137	.100	.124	.115	.133	.126	.134
14 to 16	.097	.092	.106	.064	.184	.116	.095	.103
16 to 18	.033	.063	.088	.115	.058	.078	.078	.082
18 to 20	.112	.059	.085	.103	.094	.077	.083	.093
20 to 22	.108	.112	.070	.087	.082	.092	.101	.106
22 to 24	.082	.086	.112	.098	.111	.134	.126	.144

* Pounds of gain per pound of feed consumed.

Table 33.—Efficiency of feed utilization, third year.*

AGE IN WEEKS	PER CENT DRY SKIMMILK							
	None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2	.366	.400	.390	.390	.409	.392	.390	.416
2 to 4	.334	.332	.325	.327	.342	.345	.319	.304
4 to 6	.326	.322	.324	.341	.331	.327	.359	.333
6 to 8	.260	.280	.276	.266	.266	.263	.276	.272
8 to 10	.250	.237	.236	.244	.258	.239	.229	.222
10 to 12	.203	.237	.203	.215	.205	.205	.202	.220
12 to 14	.197	.170	.197	.196	.204	.185	.226	.214
14 to 16	.143	.149	.117	.140	.125	.143	.142	.144
16 to 18	.106	.094	.111	.095	.105	.105	.133	.100
18 to 20	.086	.102	.090	.065	.089	.066	.068	.073
20 to 22	.068	.077	.106	.088	.089	.070	.088	.072
22 to 24	.104	.130	.100	.119	.114	.141	.115	.126

* Pounds of gain per pound of feed consumed.

Table 34.—Efficiency of dry skimmilk utilization, first year.*

AGE	PER CENT DRY SKIMMILK						
	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2 weeks	.52	1.12	.74	.45	.75	.83	.59
Day-old to 4 weeks	0	.40	.61	.46	.54	.45	.39
Day-old to 6 weeks	1.85	1.16	.92	.66	.59	.51	.49
Day-old to 8 weeks	0	.20	.32	.40	.29	.27	.28
Day-old to 10 weeks	0	.31	.39	.10	.25	.24	.27
Day-old to 12 weeks			.20	.14	.17	.11	.18
Day-old to 14 weeks			.17	.07	.09	.08	.17
Day-old to 16 weeks			.09	.02	0	.03	.12
Day-old to 18 weeks			.04	.01			.09
Day-old to 20 weeks			.04				.07
Day-old to 22 weeks							.01
Day-old to 24 weeks							

* The gain of the no-milk group in each interval was used as the base value for computing the excess gain of each of the remaining seven groups. The excess gain divided by the amount of dry skimmilk consumed yielded the *efficiency value—the number of pounds of excess gain per pound of dry skimmilk*. Blank spaces indicate that the particular groups gained less during those periods than did the no-milk group.

Table 35.—Efficiency of dry skimmilk utilization, second year.*

AGE	PER CENT DRY SKIMMILK						
	1.25	2.5	3.75	5.0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2 weeks		.47	.84	.30	.57	.15	.59
Day-old to 4 weeks	.29	.22	.90	.69	.75	.30	.65
Day-old to 6 weeks	.27	.68	.43	.60	.61	.11	.52
Day-old to 8 weeks	1.27	.85	.33	.63	.60	.10	.52
Day-old to 10 weeks	.72	.82	.33	.50	.53	.05	41
Day-old to 12 weeks	.73	.75	.30	.37	.41		.32
Day-old to 14 weeks	.61	.38	.18	.09	.35		.26
Day-old to 16 weeks	.45	.44		.37	.35		.23
Day-old to 18 weeks	.76	.70	.33	.40	.41	.10	.29
Day-old to 20 weeks	.02	.50	.27	.29	.28	.04	.23
Day-old to 22 weeks	.07	.24	.15	.18	.21	.01	.20
Day-old to 24 weeks	.14	.38	.21	.25	.30	.09	.29

* See note, table 34.

Table 36.—Efficiency of dry skimmilk utilization, third year.*

AGE	PER CENT DRY SKIMMILK						
	1.25	2.5	3.75	5:0	6.25	7.5	8.75
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Day-old to 2 weeks	2.85	1.39	.92	.73	.65	.60	.61
Day-old to 4 weeks	.96	.82	.60	.65	.57		
Day-old to 6 weeks	.46	.97	.74	.48	.65	.30	.23
Day-old to 8 weeks	1.13	1.11	.55	.44	.66	.32	.32
Day-old to 10 weeks	1.08	.99	.43	.50	.64	.24	.25
Day-old to 12 weeks	1.24	.87	.44	.40	.53	.22	.23
Day-old to 14 weeks	.35	.67	.38	.39	.39	.19	.22
Day-old to 16 weeks	.41	.35	.35	.27	.32	.18	.20
Day-old to 18 weeks	.12	.34	.25	.21	.27	.19	.15
Day-old to 20 weeks	.34	.34	.12	.19	.18	.13	.10
Day-old to 22 weeks	.50	.57	.24	.24	.18	.18	.12
Day-old to 24 weeks	.76	.44	.26	.24	.25	.18	.13

* See note, table 34.

Table 37.—Per cent mortality.

AGE IN WEEKS	PER CENT DRY SKIMMILK None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
Growing period, first year								
Day-old to 12	3.3	2.1	1.7	2.3	.6	3.4	.6	1.1
12 to 22	1.3	1.4		4.1		2.6	2.7	
Total*	4.6	3.5	1.7	6.4	.6	6.0	3.3	1.1
Laying period, first year								
22 to 72*	26.0	22.0	30.0	34.0	34.0	28.0	32.0	34.0
Growing period, second year								
Day-old to 12	2.3	3.6	8.3	4.7	4.7	9.1	9.4	4.8
12 to 22	4.9	3.6	2.5	3.6	1.3		1.3	1.2
Total*	7.2	7.2	10.8	8.3	6.0	9.1	10.7	6.0
Laying period, second year								
22 to 72†	46.0	60.0	44.0	56.0	56.0	26.0	32.0	26.0
Growing period, third year								
Day-old to 12	9.0	3.8	4.1	12.8	7.1	2.9	1.8	6.8
12 to 22			1.2		1.2	2.4		
Total*	9.0	3.8	5.3	12.8	8.3	5.3	1.8	6.8
Laying period, third year								
22 to 72*	30.0	44.0	32.0	34.0	42.0	26.0	32.0	26.0

* Differences not significant.
† Differences highly significant.

Table 38.—Shrinkage in weight of males shipped alive to market, first year.

	PER CENT DRY SKIMMILK None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
Single Comb White Leghorn males, 10 weeks of age								
Mean body weight in pounds when shipped	2.17	2.15	2.23	2.20	2.25	2.23	2.20	2.23
Per cent shrinkage in transit	15.7	13.0	11.7	12.3	12.4	13.5	15.0	11.7
Barred Plymouth Rock males, 12 weeks of age								
Mean body weight in pounds when shipped	2.78	2.80	3.00	2.78	2.90	2.73	2.85	2.90
Per cent shrinkage in transit	13.7	8.9	10.0	9.0	9.3	9.2	10.5	12.8

Table 39.—Mean biological values of experimental rations.†

TRIAL	PER CENT PROTEIN IN DIET	EGG PROTEIN CONTROL	PER CENT DRY SKIMMILK IN EXPERIMENTAL RATION None	1.25	2.5	3.75	5.0	6.25	7.5	8.75
1	9	94.9	74.4	79.4	73.0	74.0	76.6	75.2	75.7	78.0
1	14.5	79.6	67.8	66.3	62.8	67.0	68.2	69.6	65.4	64.0
2	9	97.2	75.2	75.1	72.5	72.5	76.8	76.6	75.3	75.7
2	14.5	72.5	62.9	62.4	58.9	60.4	63.3	64.4	58.0	58.4
Mean at 9 per cent level*		96.1	74.8	77.3	72.7	73.3	76.7	75.9	75.5	76.8
Mean at 14.5 per cent level‡		76.1	65.4	64.3	60.8	63.7	65.7	66.9	61.7	61.2

† The biological value is the percentage of the protein intake that is actually utilized by the animal.
* Minimum difference between means to be significant: 3.0 per cent.
‡ Minimum difference between means to be significant: 3.5 per cent.

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The Disintegration of Erythrocytes and Denaturation of Hemoglobin by High Pressure.

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[Plates VIII.–X.]

No data appear in the literature on the general effects of high hydrostatic pressure on mammalian blood, although Bridgman and Conant † have reported that Dr. A. R. Davis observed the coagulation of blood at high pressure several years prior to their experiments on the apparent denaturation of carboxyhemoglobin at pressures of 9000 atmospheres.

We have observed recently several interesting pressure effects in bovine blood which have not been reported hitherto. These experiments were performed in the high pressure laboratory of the Department of Physics with apparatus similar to that of Bridgman and Conant. The samples of blood, prepared as described below, were subjected to constant pressures that ranged from 3500 to 13,000 atmospheres at room-temperature which varied from 27° to 29° C. Two series of experiments are described and the results briefly summarized.

I.

Initial tests were made with sterile oxalated, bovine blood. Five to seven c.c. of blood were introduced into a glass tube by evacuating the tube under blood within a bell-jar and then admitting air. The tube was then inverted in mercury in a steel cylinder and the assembly placed in the testing chamber of the high pressure apparatus.

A typical experiment was one run at 3500 atmospheres for 3 hours with the following results :—erythrocyte count decreased from 7,300,000 to 800,000, and leucocytes decreased from 6700 to 800 per cu. mm., both approximately in the same proportion ; both types of cell appeared distorted in a microscopic field of 1000 x ; NaCl crystals, and possibly crystals of other salts, were clearly visible ; oxygen binding capacity decreased by 41 per cent. as measured by the Van Slyke method. Pressure treatment of a sample for 1 hour at 3500 atmospheres gave results roughly

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[†] P. W. Bridgman and J. B. Conant, Proc. N.A.S. xv. p. 680 (1929).

comparable with those of the 3 hour exposure. The oxygen binding capacity in this case decreased by 16 per cent.

A 6 hour exposure at 3500 atmospheres, as well as a $3\frac{1}{2}$ hour exposure at 13,000 atmospheres, coagulated the samples of blood into reddish-brown rubbery masses. Microscopic examination of the precipitate revealed no erythrocytes, very few distorted leucocytes, no crystallization, and a jelly-like material that was without apparent structure but containing dispersed nuclei from leucocytes.

II.

The second series of tests were made on blood-cells separated from freshly drawn oxalated bovine blood by means of a centrifuge, and with aqueous solutions of hemoglobin in order to corroborate the results of (I.) under more simple experimental conditions. The aqueous solutions contained approximately 50 per cent. normal concentration of hemoglobin. The samples in these tests were placed in paraffin-lined steel tubes without filling by evacuation. Paraffin-oil was used to separate the pressure-transmitting liquid from the samples. The experiments were conducted at constant pressures of 5000 atmospheres for intervals ranging from $\frac{1}{2}$ to 2 hours.

The blood-cells in each case were entirely coagulated, giving a material similar to that observed previously (I.) when coagulation was obtained at higher pressures and for longer times. Apparently the proteins of the red cells, due either to their higher concentration or to their nature, were more easily coagulated than the proteins of the plasma. No crystallization occurred in this series of experiments.

The aqueous solutions of hemoglobin were treated with the same pressures for periods of 2 and 4 hours respectively. The coagulation, while not complete, was similar in structure to that observed for bovine blood. It appeared similar to that obtained by treating control solutions with ethyl alcohol and with diethyl ether. Figs. 1, 2 (Pl. VIII.) and fig. 3 (Pl. IX.) are photomicrographs (1000 ×) of blood-cells exposed at 5000 atmospheres for $\frac{1}{2}$ hour, aqueous solution of hemoglobin exposed at 5000 atmospheres for 2 hours, and control hemoglobin solution treated with alcohol, respectively. Bridgman and Conant believe that carboxyhemoglobin is " denatured " by pressure treatment similar to the denaturation obtained by the action of alcohol on carboxyhemoglobin. The result of our experiments lead us to much the same conclusion for the effect of pressure on hemoglobin.

To observe the effect of pressure on the erythrocytes more clearly a small sample of blood-cells was suspended in Hayem's solution and then subjected to pressure treatment of 5000 atmospheres for 1 hour. Fig. 4 (Pl. IX.) and fig. 5 (Pl. X.) are photomicrographs (1000 ×) of the cells before and after pressure treatment respectively, showing the drastic effects of pressure treatment.

In summarizing the results of the two series of tests it is interesting to observe that crystallization of salt occurred when the coagulation of protein was incomplete. This would indicate that salts were being removed from solution by reacting with other substances present under pressure, and agree with the views of other investigators * who believe that in a denaturation process the denaturation may inhibit crystallization.

The question arises whether the observed disintegration of blood-cells is due to the mechanical effects of pressure or chemical effects under pressure. This was partly explained by results obtained when an unexpected leak developed in the pressure apparatus during one of the tests. After an exposure of about 1 minute at 2500 atmospheres a leak in pressure connexions was noticed, and in an attempt to maintain the pressure by rapid pumping considerable fluctuation in pressure necessarily occurred. Microscopic examination of the blood then showed numerous salt crystals in the field but little change in the appearance of the cells. The nuclei of the leucocytes suffered elongated distortion. This test indicated to us that the effects of pressure change were not primarily mechanical ones. While the cells are clearly distorted and might be ruptured by quick release of pressure it is not likely that the pressures that we used could be responsible for the observed disintegration of cells.

As mentioned previously, one of the effects of pressure seemed to be comparable to the addition of an OH radical to the hemoglobin. This effect may involve the breaking up of polypeptide linkages $>\mathrm{N}-(\mathrm{OH})\mathrm{C}<$ into new independent groups, $>\mathrm{NH}$, $\mathrm{OC}<$ possibly accompanied by hydration. This mechanism of denaturation proposed by Wrinch † seems extremely suggestive, but we believe that pressure aids the disintegration rather than inhibits it as Wrinch predicts. The reaction under pressure appeared to be a reduction. The disintegration of erythrocytes resulted from this reaction rather than from rupture of cell structure. The decrease of O_2 binding capacity content appears to be governed by a first-order reaction, similar to the observations of Bridgman and Conant on CO.

This research was made possible by the co-operation of several of the senior author's colleagues. Dr. J. F. Shigley generously supplied samples of bovine blood, Dr. M. L. Willard loaned the camera for the photomicrographs, and Dr. A. K. Anderson not only placed his equipment at our disposal but also assisted in criticizing the manuscript.

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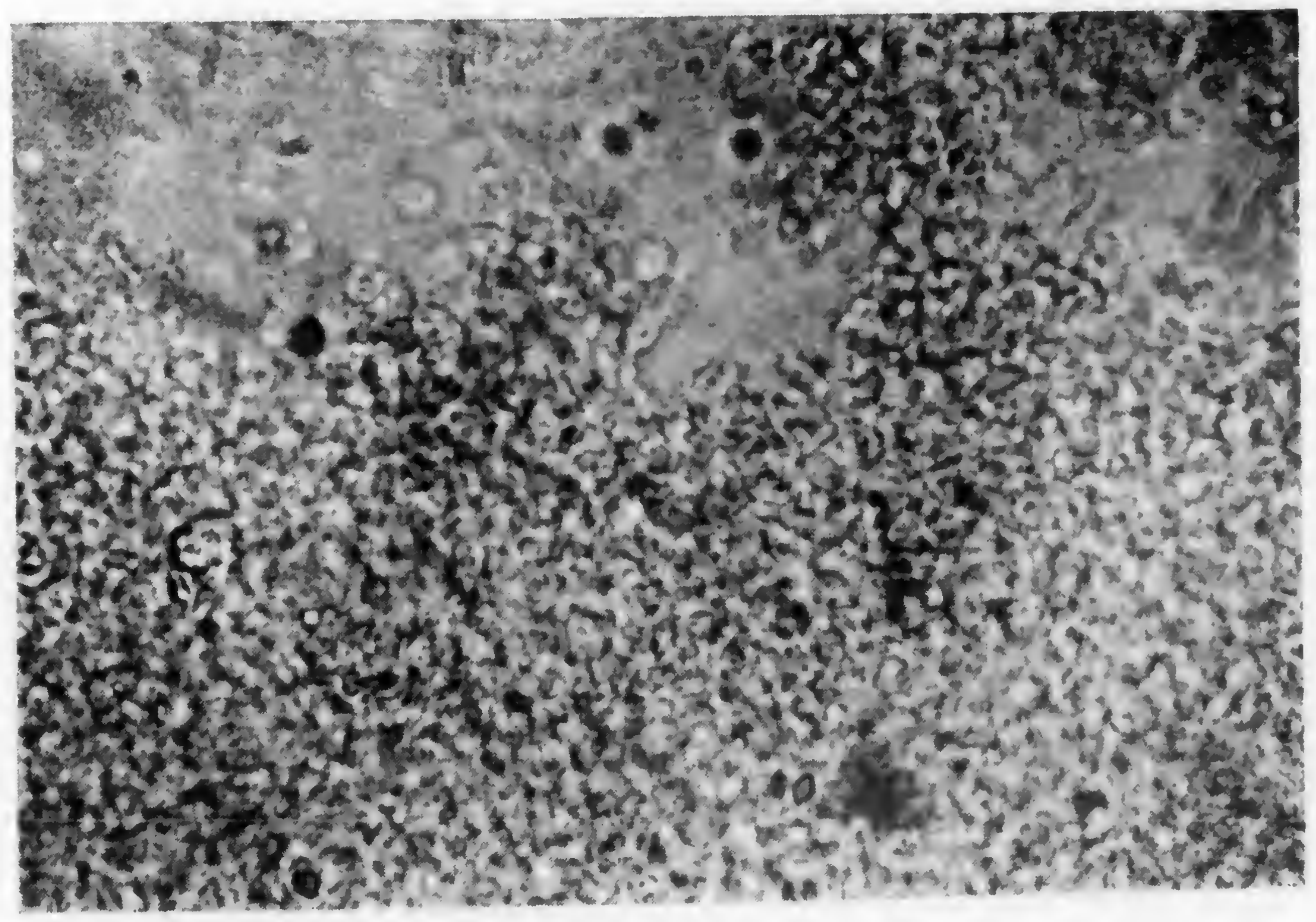
* D. M. Wrinch, Phil. Mag. xxv. p. 705 (1938).

† *Loc. cit.*

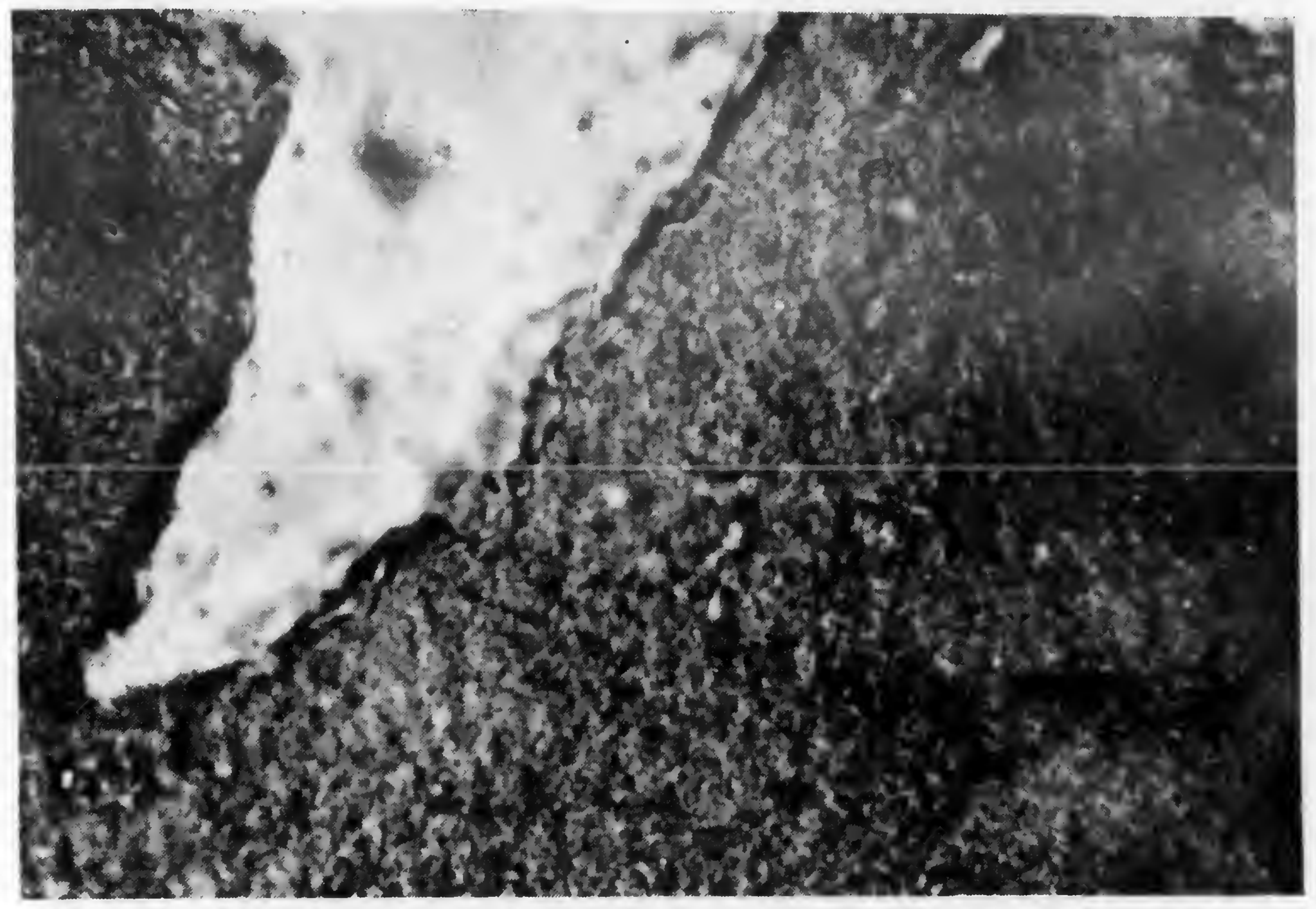
R. B. DOW & J. E. MATTHEWS, Jr. Phil. Mag. Ser. 7. Vol. 27. Pl. VIII.

FIG. 1.



Blood-cells after pressure treatment at 5000 atmos. for $\frac{1}{2}$ hour. Magnification 1000 ×.

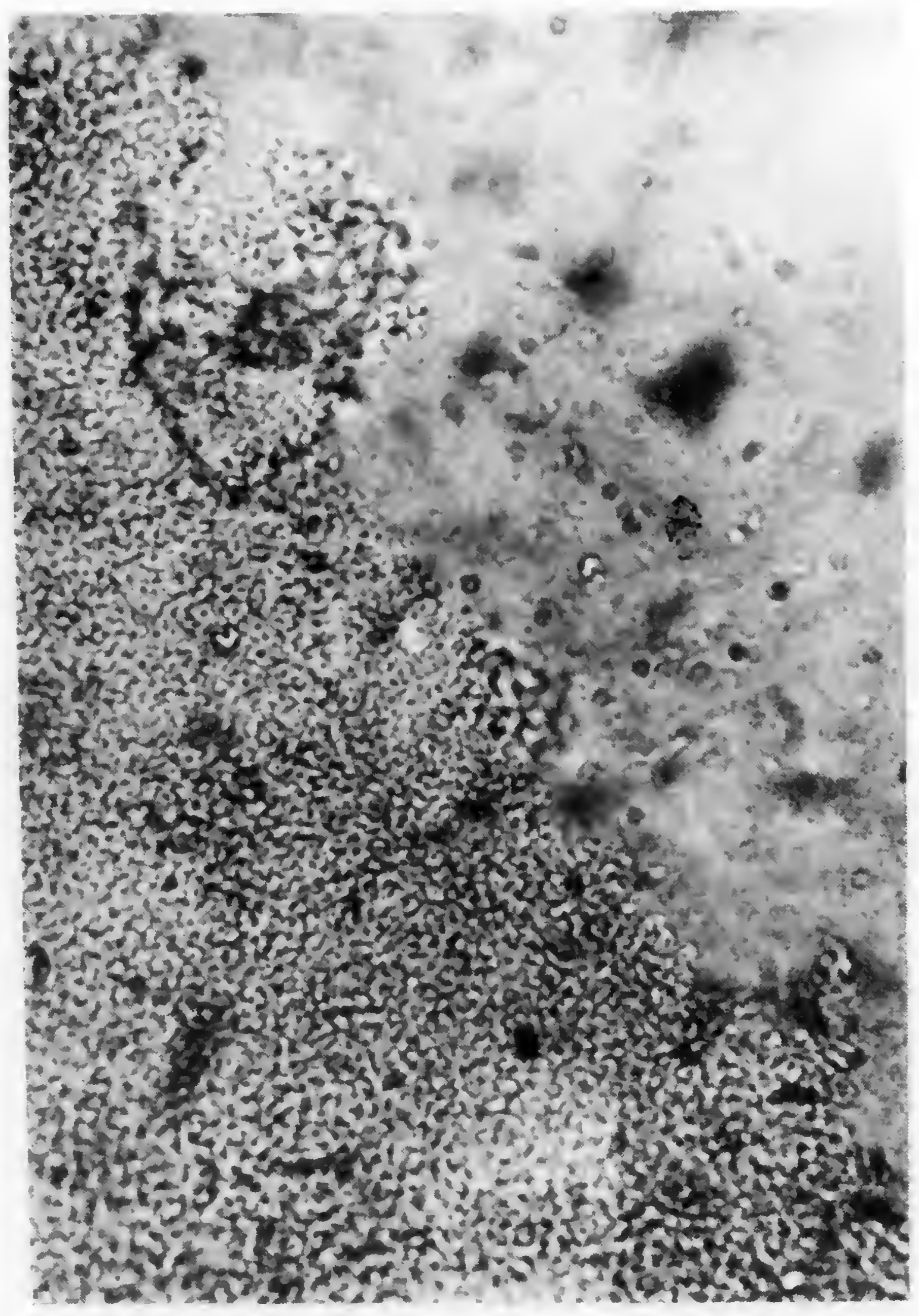
FIG. 2.



Aqueous solution of hemoglobin after pressure treatment at 5000 atmos. for 2 hours. Magnification 1000 ×.

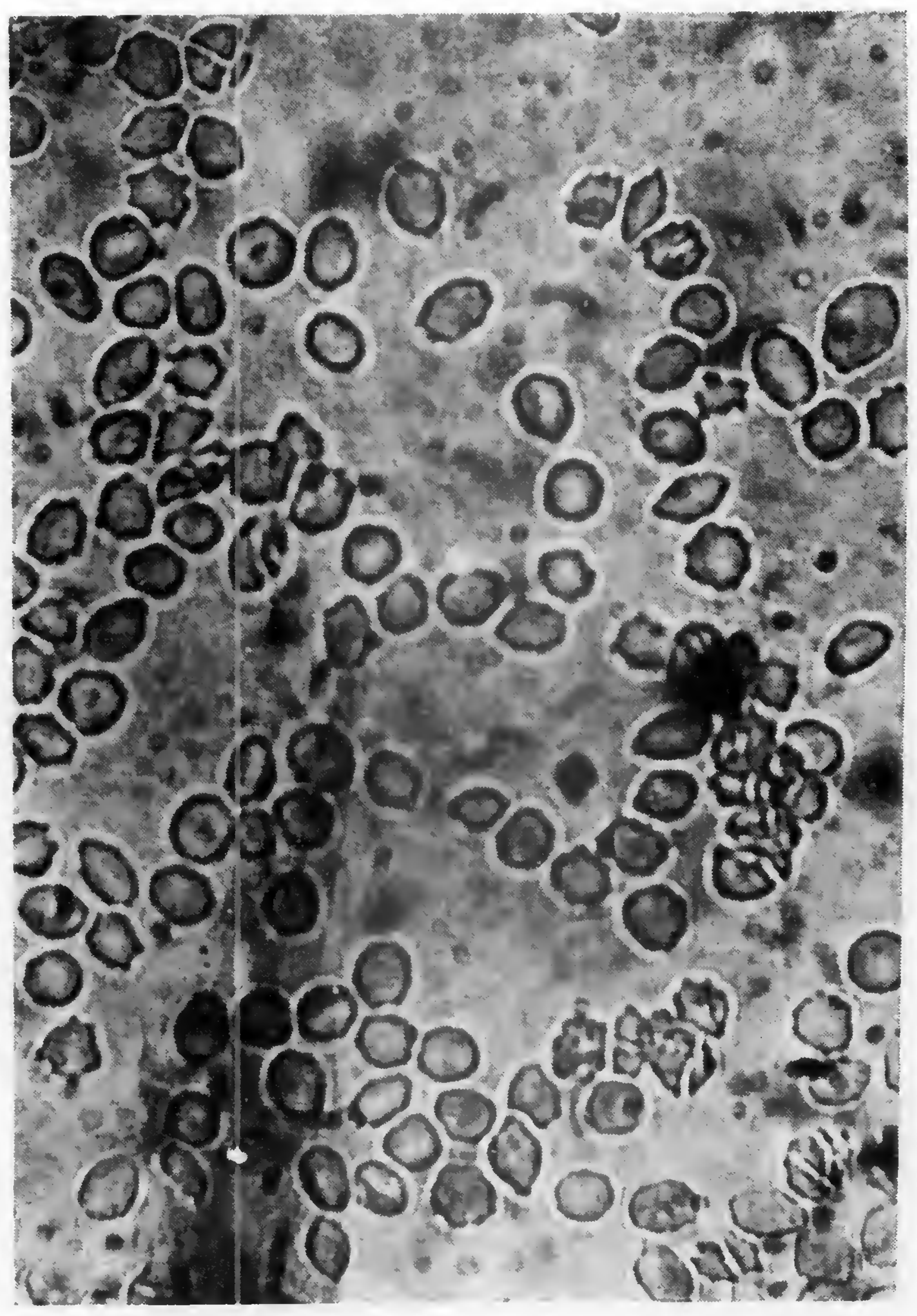
R. B. DOW & J. E. MATTHEWS, Jr. Phil. Mag. Ser. 7. Vol. 27. Pl. IX.

Fig. 3.



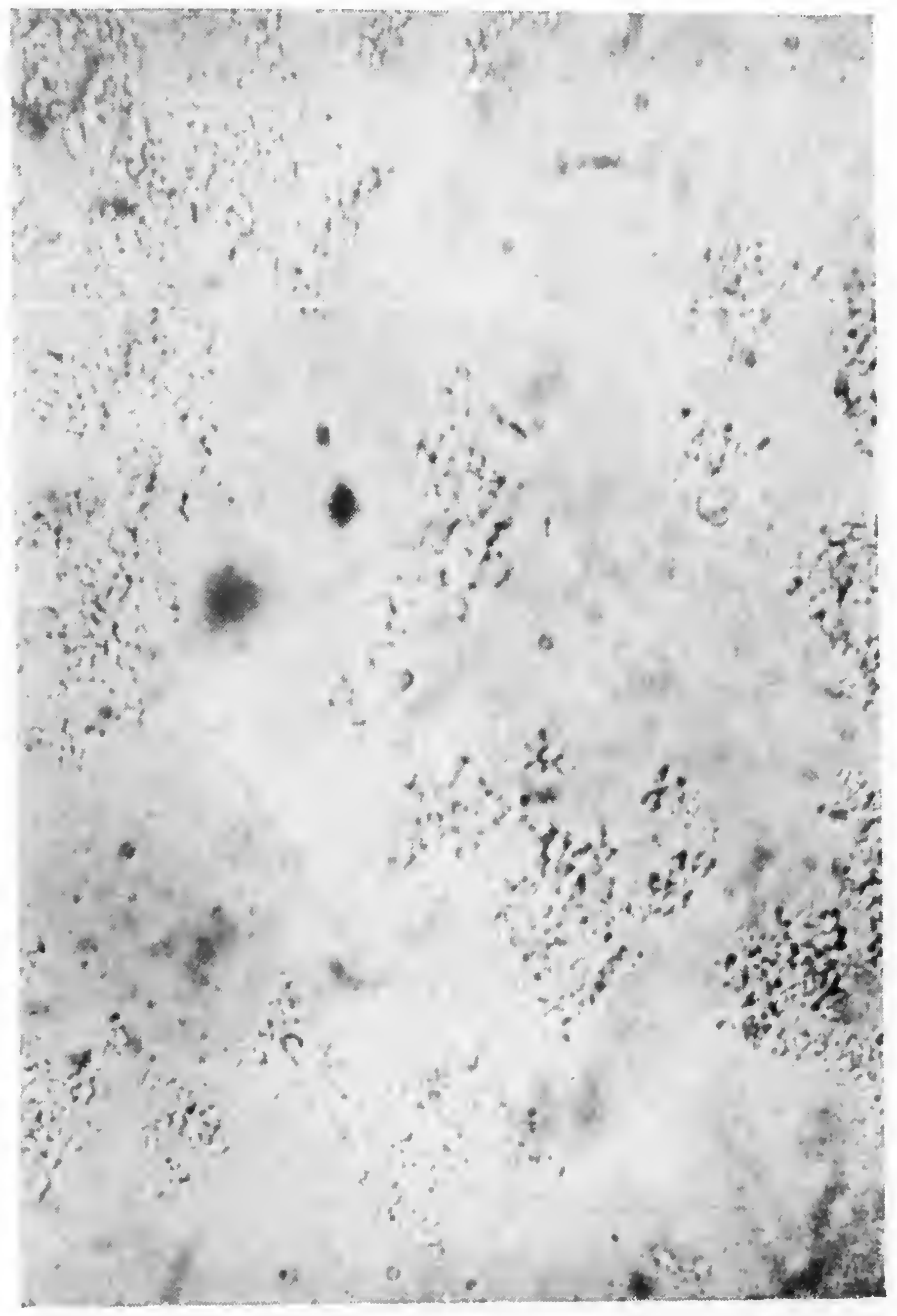
Aqueous solution of hemoglobin after treatment with ethyl alcohol. Magnification 1000 ×.

Fig. 4.



Erythrocytes of normal bovine blood suspended in Hayem's solution. Magnification 1000 ×.

FIG. 5.



Erythrocytes disintegrated by pressure treatment of 5000 atmos. for 1 hour. Magnification 1000 ×.

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No. 2317, pages 490–491.

NICOTINIC ACID IN SWINE NUTRITION

IN November, 1938, a herd of pigs in northwestern Pennsylvania was reported to the agricultural extension specialists of the Pennsylvania State College as being sick and unthrifty. These pigs were farrowed in September and weighed from twenty to forty-five pounds. Out of a total of seventy-six pigs, forty had died during October and November. The surviving pigs had stopped growing, were without appetite and were affected with diarrhoea and a dermatitis on the body and ears which had the appearance of a heavy scurf. This condition developed while the pigs were being fed a ration of corn, oats, wheat middlings, tankage (34 per cent. protein) and a limited amount of skimmed milk. They had access to good grass pasture which had never previously been used for swine.

At the time the case was reported these pigs were accustomed to huddle in their pens rather than to take advantage of the available pasture. When food was placed before them they would come to the trough, eat a small amount and refuse the remainder of the feed.

The condition of these animals baffled the local and district veterinarians and the case was brought to the attention of the college swine specialist. It was then noted that the symptoms of the disorder were similar to those reported by Chick and co-workers,[1] with pigs suffering from nicotinic acid deficiency on a diet consisting largely of maize. As a result of this observation seven pigs, having been selected as being in the poorest condition of any in the herd, were given 50 mg daily of nicotinic acid mixed in a minimum amount of feed to insure its complete consumption. Within twelve days a marked improvement was noted in these seven pigs. A return of appetite was apparent and increased physical activity was displayed, the pigs searching for food and having every indication of return to normal health. The dermatitis disappeared from the ears where new hair began to grow and the scurf on the back was lifted by the growth of the new hair.

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[1] *Biochem. Jour.*, 32: 10–12, 1938.

Nicotinic acid was then given to the entire herd in 50 mg daily amounts mixed in the feed, and complete recovery was made by all the pigs as noted with the previous seven. The dramatic response to nicotinic acid treatment is similar to that described by Chick. The feeding of nicotinic acid was continued for two weeks. As pasture was not available ground alfalfa and cod liver oil were then included in the ration.

Within six weeks the entire herd had completely recovered, growth having been resumed and the entire lot of pigs appearing to be normal.

This is believed to be the first time that a nutritional disorder simulating swine pellagra has been reported as occurring under conditions of agricultural practice. The effectiveness of the administration of nicotinic acid in alleviating the condition adds further proof as to its identity. It is not believed that this condition occurs in practice to any great extent, especially as affecting entire herds as in this case. However, individual pigs are sometimes seen whose condition resembles that described above.

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No. 2320, pages 566–567.

BACTERIAL LEAF-SPOT DISEASES[1]

AN investigation has been made of the leaf-spot of Pennsylvania cigar-leaf tobacco, commonly known as "wildfire." Evidence obtained in these laboratories indicates that the organism associated with the leaf-spot, *Phytomonas tabaca,* is a transitory physiological adaptation of the common saprophyte, *Pseudomonas fluorescens.* This organism, in various physiological adaptations, is found in large numbers on normal tobacco. It is apparent from the data obtained in these investigations that all Pennsylvania cigar-leaf tobacco is exposed to this organism throughout the growing and ripening period and that infection in the field is due not to the mere presence of the organism, which is ubiquitous, but to improper host nutrition.

Single cell isolations of various adaptations of the organism have been the subject of physiological and serological studies. The particular adaptation, or what might be termed by the plant pathologist "relative virulence" has been found to vary greatly according to the source of the isolation. Distinct physiological characteristics have been found to be typical of these various isolates. Rapid changes may be made in the laboratory at will, both in the direction of increased and decreased virulence. Serologically, it appears that the "virulence" of the organism is associated with the specific nature and amount of the capsular material of the cell. Although the particular adaptation of the organism is probably related to severity and rapid spread of infection in those cases in which the tobacco plant is suffering from improper nutrition, the evidence indicates on the other hand that the most "virulent" adaptations of the organism are unable to cause economic loss in those cases in which the nutrition of the plant is satisfactory, other things being equal.

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[1] Authorized for publication on March 27, 1939, as paper No. 898 in the journal series of the Pennsylvania Agricultural Experiment Station.

The normal tobacco plant of the cigar-filler type is very resistant to infection of economic severity during the growing season. Leaves of such a plant ordinarily contain at maturity between 3 and 4 per cent. nitrogen and between 4 and 5 per cent. potassium. Changing agricultural practices of the past quarter century have resulted in the general production of an abnormal tobacco from the standpoint of nitrogen and potassium content.

Investigations have shown that, contrary to the opinions expressed in publications by other workers, it is not the exact nitrogen level within reasonable limits that is important but the ratio of nitrogen to certain minerals within the plant and, of extreme importance, the stage in which high nitrogen uptake occurs.

Any condition which leads to the accumulation in the plant of a greater quantity of nitrogen than potassium results in poor quality and susceptibility to leaf spot troubles. It is not to be inferred from this that potassium is the only other element which must be available to the plant in order to insure quality and disease resistance. Potassium is, however, the element needed in greatest amount and apparently most frequently deficient in the Pennsylvania area.

It is not during the period of active growth but during the period of ripening that faulty agricultural practices may lead to the greatest susceptibility to leaf-spot. Our investigations have shown that, regardless of the nutrition of the plant prior to this period, the uptake of significant quantities of nitrogen during the ripening period results in an appreciable lowering of the normal resistance of the plant to wildfire. In Pennsylvania certain practices are necessary which accentuate the abnormal condition brought

about by significant nitrogen uptake during ripening and greatly increase the susceptibility of those plants taking up large quantities of nitrogen during the ripening period. These include "topping" and "suckering" which take from the plant the possibility of utilizing large amounts of nitrogen at this period in the proliferation of new tissue. It is significant that wildfire was not a problem in Pennsylvania a number of years ago when agricultural practice precluded the possibility of any appreciable ammonification and nitrification at the ripening period.

Practices in Pennsylvania which have been found to be largely responsible for the severity of leaf-spot infections are, in the order of their importance: (1) the incorporation of large quantities of organic nitrogen in the tobacco soils, a portion of which in some seasons may remain to be ammonified during the ripening period; (2) the use of too much nitrogen and insufficient potash in the fertilization treatment; (3) the use of excessive amounts of lime, which interferes with the normal potassium uptake of the plant; and (4) in some cases poor tilth, which also interferes with mineral uptake.

Practices tending to produce a normal resistant plant of high quality and good yield are as follows: (1) the use of another crop such as corn between clover and tobacco in the rotation to reduce the amount of organic nitrogen in the soil; (2) preliminary rotting of all manure used on tobacco in order to avoid the risk of adding large quantities of available energy to the soil; (3) the use of liberal applications of well-rotted manure to improve tilth and aid potassium uptake; (4) the use of suitable applications of a well-balanced fertilizer in which the amount of organic nitrogen is less than the amount of inorganic nitrogen; and (5) suitable cultivation to insure maximum uptake of potassium and other essential nutrient materials.

In the course of these investigations it was considered advisable to study physiologically and serologically several other members of the genus *Phytomonas*, associated with leaf-spots. The data obtained indicate that other members of this genus studied are merely temporary physiological adaptations of *Pseudomonas fluorescens*. A brief study of predisposing factors in these diseases further emphasizes the similarity of bacterial leaf-spot diseases and the relation of the pathological condition to the improper nitrogen-mineral nutrition of the host. The results of these investigations will be published in detail in a Bulletin of the Pennsylvania Agricultural Experiment Station.

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CENTRAL PENNSYLVANIA BRANCH

Annual Spring Meeting, The Gilliland Laboratories, Marietta, Pa., May 13, 1939

Plant Nutrition and Disease Resistance. *J. Naghski, R. G. Harris, D. E. Haley and J. J. Reid*, The Pennsylvania Agricultural Experiment Station.

A study has been made of the relation between variations in the nitrogen /mineral uptake of plants and variations in resistance to disease. It has been a common assumption that, other things being equal, the higher the nitrogen level of the plant, the greater the susceptibility of the plant to disease. Data obtained by the authors indicates that this is not the true relation between nitrogen uptake and disease susceptibility.

The results indicate that it is not a question of how much nitrogen a plant absorbs within reasonable limits, that determines susceptibility, but the stage in the life of the plant when significant quantities of nitrogen are absorbed.

Tobacco plants grown with adequate mineral fertilization may mature with a nitrogen content in excess of four per cent and show no significant loss in resistance to leafspot diseases if the nitrogen is supplied without material fluctuations during the stage of active growth. On the other hand it has been found that tobacco plants may show extreme susceptibility to leafspot diseases with a nitrogen content of less than two per cent, if the nitrogen is supplied erratically with significant amounts available during the ripening stage.

It is considered inadvisable, therefore, to employ fresh animal manure, old legume residues and other sources of nitrogen which may provide a fluctuating and late supply of this element in the fertilization of tobacco and other short season crops.

Dissimilation of Glucose by Members of the Genus Bacillus. *F. H. Gallagher and R. W. Stone*, The Pennsylvania State College.

Studies were made of the dissimilation of glucose by *Bacillus subtilis*, *Bacillus mesentericus*, and *Bacillus vulgatus*. All species showed phosphorylation in the early stages of fermentation. Analysis of the fermentation showed the presence of acetic acid, butyric acid, ethyl alcohol, acetyl methyl carbinol, and carbon-dioxide, in all cases. These compounds were determined quantitatively.

With M/50 sodium fluoride *Bacillus subtilis* showed a higher yield of acetic acid than that obtained in the control. With M/25 sodium fluoride there was a further increase in the amount of acetic acid formed, and a decrease in acetylmethylcarbinol production. *Bacillus vulgatus*, in the presence of M/100 and M/50 sodium fluoride, and *Bacillus mesentericus*, in the presence of M/50 sodium fluoride, gave similar results.

All three species gave comparative results as to phosphorylation, types of end products, and amounts of these products.

The Photolometer and Its Use for the Quantitative Determination of Nitrogen. *Paul E. Portner*, Gilliland Laboratories, Inc.

Microorganisms Attacking Medium Weight Lubricating Oils. *A. G. C. White and R. W. Stone*, The Pennsylvania State College.

The Relation of the Soluble Specific Substance to Virulence and Specificity in Bacterial Leafspot Organisms. *R. G. Harris, J. Naghski, M. A. Farrell and J. J. Reid*, The Pennsylvania Agricultural Experiment Station.

Laboratory animals have been immunized by the authors with cultures of *Pseudomonas fluorescens* and the cultures then cultivated in the presence of the homologous antiserum until "R" forms were secured. Animals were then immunized with the "R" forms and the "R" cultures were cultivated in the presence of the homologous antiserum and killed cells of *Phytomonas tabaci*. "S" forms were obtained in this manner which proved to be culturally and serologically identical with strains of *Phyt. tabaci*. Studies of infective ability showed these "S" forms to be identical with *Phyt. tabaci* in the nature of the lesion produced on the tobacco plant.

In a similar manner "R" forms of *Phyt. tabaci* were secured and cultivated in the presence of the homologous antiserum and killed cells of *Ps. fluorescens*. The "S" forms produced in this manner were culturally and serologically identical with the strains of *Ps. fluorescens* which had been killed and used in the culture medium. These "S" forms were unable to produce wildfire as proved by infective studies.

Agglutinin absorption studies showed the "R" strains produced from *Ps. fluorescens* and *Phyt. tabaci* to be serologically identical.

Work with several other members of the genus *Phytomonas*, including *Phyt. angulata, Phyt. cerasi, Phyt. primulae*, and *Phyt. vignae*, showed the same relationship to exist between these organisms and *Ps. fluorescens*.

It is concluded that specificity and virulence are associated with the nature of the soluble specific substance in this group of organisms.

SOME EFFECTS OF SULFAPYRIDINE ON PNEUMOCOCCUS TYPE I. *Roger D. Reid,* Laboratories of Bacteriology, Johns Hopkins Medical School, Baltimore.

In vitro and *in vivo* experiments with the pneumococcus Type I in the presence of sulfapyridine indicated that the drug had no appreciable effect on the phagocytosis of pneumococci. There were no changes in morphology or somatic elements of the cell observed. No dissociation or changes in colony appearance were detected after growing the organisms in the presence of 10 mgm. % of the drug or when the drug was added to the medium used for plating the organisms. The drug did not affect the capsule either *in vivo* or *in vitro* and the "quellung" reaction was not interfered with.

A bacteriostatic and slight bactericidal effect was noted and it is presumed that the bacteriostatic action is responsible for the beneficial effects of the drug in pneumococcus infection.

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Vol. 24, No. 11, Page 1209, August, 1939. (Printed in the U. S. A.)

FREE CHLORINE AS A SOURCE OF ERROR IN BLOOD SUGAR DETERMINATIONS*

ARTHUR K. ANDERSON, PH.D., AND ISADORE ZIPKIN, B.A., STATE COLLEGE, PA.

RECENTLY we have experienced a difficulty in determining blood sugar by the Folin and Wu[1] method which we feel should be called to the attention of those working with this or similar methods. The difficulty was a rapid fading of the blue color upon dilution with water before making the colorimeter reading. Within five minutes the solution became colorless. Fading occurred even when a freshly prepared 1:4 dilution of acid molybdate solution was used as the diluting agent. After considerable search for the difficulty, it was finally found that the fading was due to the reoxidation of the blue compound to a colorless compound by free chlorine in the distilled water. The distilled water used was found to contain 0.2 part per million of free chlorine, as measured by the ortho-tolidine method.[2] Another source of distilled water was found to contain 0.1 part per million of free chlorine. This water did not cause complete fading, but fading was noticeable. When chlorine-free water was used, no fading was noted, the solutions retaining their blue color overnight.

It is felt that wherever chlorinated water is used in the preparation of distilled water, the danger of free chlorine distilling over is eminent. This is especially true where adequate control of the chlorination process is not maintained.

Although the effect of free chlorine has been noted only in connection with the Folin and Wu method for determining blood sugar, it is felt that it would interfere with other similar methods for sugar and also with any other method where color is the result of a reduction process.

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*From the Department of Agricultural and Biological Chemistry, the Pennsylvania State College, State College.

Received for publication November 1, 1938.

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Reprinted from Analytical Edition, INDUST

Determinatio
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DURING the past few years it has
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tions on various surfaces sprayed with copp
surfaces upon which the copper was dep
leaves and fruits of apples, cherries, and
tain synthetic surfaces, particularly Py
present being used in this laboratory for
on the deposition and retention of sprays.

A number of methods have been inv
have been selected as the most satisfactor
conditions. Since an increasing number
fields of insecticides and fungicides are tur
to the copper compounds, and since few
the determination of the small amounts
upon plant surfaces have been described i
following two methods are presented
modifications of well-known analytical p
been used with complete success in this l
time.

Method A

When copper is present in the sample i
than 2 mg., the most accurate and conve
termination is by direct weighing after e
platinum electrodes. The determination
(under 50 mg.) is considerably more diffi
the ordinary electrodeposition as followed
per ores or alloys. The procedure fou
leaf samples which have received one or m
copper sprays is as follows:

A sample of from 2 to 20 grams of the dr

TABLE I. COPPER PRESENT IN A
AS DETERMINED BY ELECT

Type of Leaf	Treatment
Apple	1 application of $Cu_3(PO_4)_2$
	1 application of Cupro-K
	2 applications of copper ars
Cherry	1 application of $Cu_3(PO_4)_2$
	2 applications of basic copp

TABLE II. RECOVERY OF ADDED
WASH SOLUT

Copper Added	
Mg.	
0.050	0
0.075	0
0.100	0
0.150	0

sults is shown in Table II. Each f
plicate determinations. Replicat
agree within 2 microamperes (abou

If a photoelectric colorimeter is n
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In this case the color should be de
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Reprinted from Analytical Edition, INDUSTRIAL AND ENGINEERING CHEMISTRY, *Vol. 11, Page 494, September 15, 1939*

Determination of Small Amounts of Copper in Spray Residues

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DURING the past few years it has been necessary for the author to make a large number of copper determinations on various surfaces sprayed with copper fungicides. The surfaces upon which the copper was deposited included the leaves and fruits of apples, cherries, and tomatoes, and certain synthetic surfaces, particularly Pyralin, which is at present being used in this laboratory for most of the studies on the deposition and retention of sprays.

A number of methods have been investigated, and two have been selected as the most satisfactory under the present conditions. Since an increasing number of workers in the fields of insecticides and fungicides are turning their attention to the copper compounds, and since few rapid methods for the determination of the small amounts of copper present upon plant surfaces have been described in the literature, the following two methods are presented briefly. These are modifications of well-known analytical procedures and have been used with complete success in this laboratory for some time.

Method A

When copper is present in the sample in amounts greater than 2 mg., the most accurate and convenient method of determination is by direct weighing after electrodeposition on platinum electrodes. The determination of small quantities (under 50 mg.) is considerably more difficult, however, than the ordinary electrodeposition as followed in the case of copper ores or alloys. The procedure found satisfactory for leaf samples which have received one or more applications of copper sprays is as follows:

A sample of from 2 to 20 grams of the dried material is ashed at a temperature not exceeding 450° C. The ash is dissolved in nitric acid (1 to 1) and transferred to a 150-ml. beaker. To this solution are added 10 ml. of a saturated solution of ammonium nitrate and 1 gram of urea, and the volume is made up to about 100 ml. The electrolysis is then carried out in the usual manner, using a platinum gauze cathode and a rotating platinum loop anode. The current between the electrodes must be much lower than is usually recommended in the methods for the electrolytic deposition of copper described in the literature, and should not exceed 0.15 ampere. Currents in excess of this amount will cause the deposition of copper oxide. The time required for complete deposition is a function of the quantity present, but for the amounts normally present on leaf samples 15 minutes is usually sufficient.

Typical results on different types of leaf material are shown in Table I.

Method B

When the total quantity of copper in the sample is less than 2 mg., it is usually not possible to weigh the metal directly with sufficient accuracy. Samples of fruits and small areas of synthetic surfaces sprayed in the laboratory usually bear less than 1 mg., and hence require a method of analysis sensitive to smaller amounts.

After thorough trials of several methods, including the chromotropic reagent method of Ansbacher, Remington, and Culp (*1*), the xanthate method (*7*), and the thiocyanate method of Elvehjem and Lindow (*4*), the method of Callan and Henderson (*2*) as modified by Cockburn and Herd (*3*) was selected as most adaptable to the present use.

The method as finally adopted in this laboratory is as follows:

The solution containing the copper is freed from organic matter, if the latter is present, by digestion with sulfuric and nitric acids. For routine analysis of fruits, it has been customary to wash the surfaces thoroughly in hot 10 per cent nitric acid solution, making the washings to volume and digesting an aliquot portion. The laboratory sprayed plates are washed in 50 per cent nitric acid and the wash solution is concentrated, made to volume, and used without digestion.

The solution, free from organic matter, is neutralized with concentrated ammonium hydroxide, and about 10 ml. are added in excess. This mixture is then boiled for a few minutes, allowed to stand for 30 minutes, filtered through a fast filter paper, and washed. The entire filtrate, or an aliquot of it, is transferred to a Nessler tube, 25 ml. of concentrated ammonium hydroxide are added, and it is made to a volume of 100 ml. The tube is then placed in a photoelectric colorimeter of the type described by Frear and Haley (*5*) or Samuel and Shockey (*6*), the light intensity is adjusted to the maximum, 1 ml. of a 1 per cent solution of sodium diethyl dithiocarbamate is added, the solution is stirred, and a second reading is taken. By calibrating the instrument with known amounts of copper, the reading in microamperes may be converted directly into milligrams of copper. A typical calibration curve is shown in Figure 1.

Accuracy of Method B

For the greatest accuracy, a sample should be selected which contains between 0.05 and 0.15 mg. of copper. Numerous recovery tests have been run with apple wash solutions containing no copper, to which have been added known amounts of copper as copper sulfate. A typical set of re-

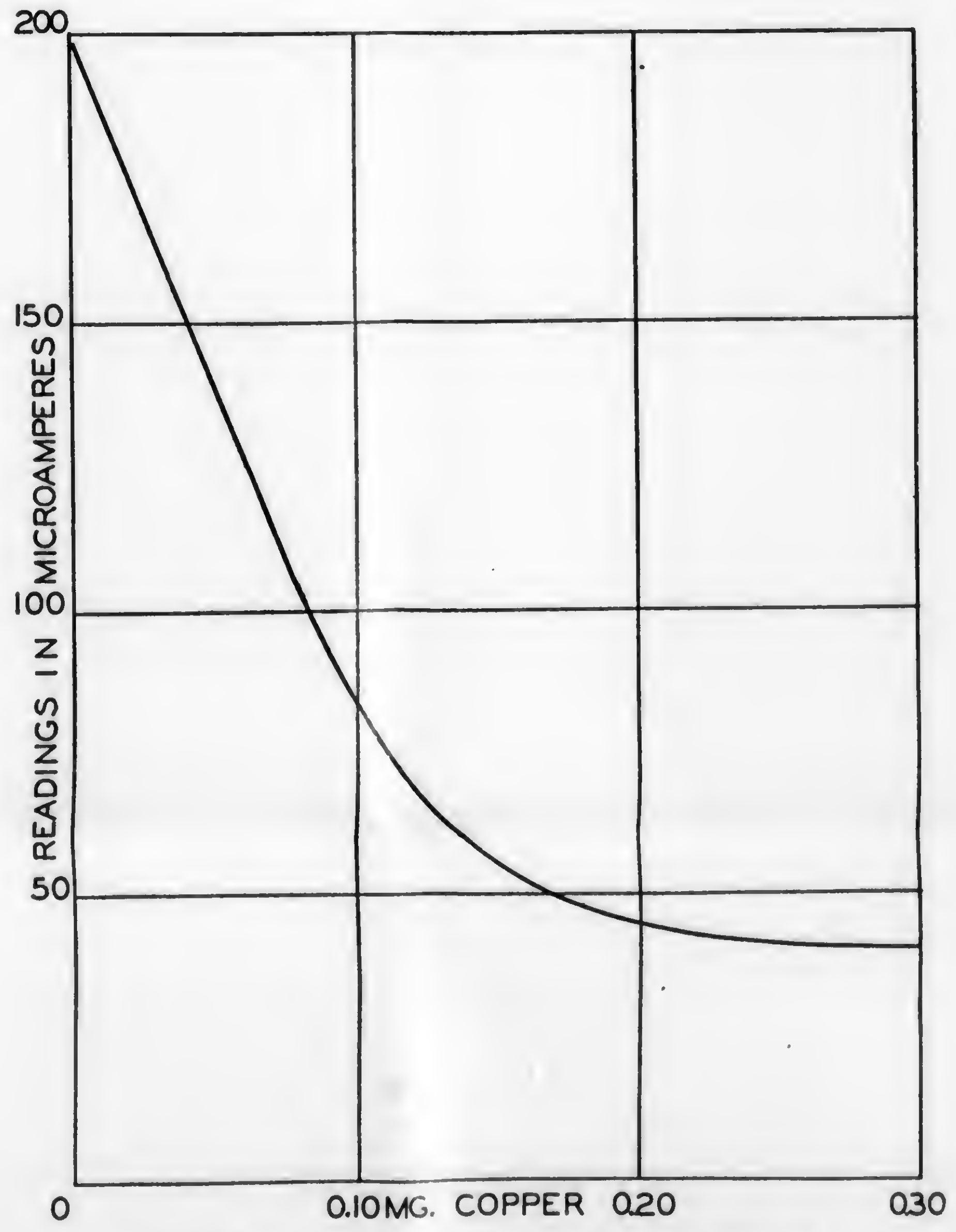

FIGURE 1. TYPICAL CALIBRATION CURVE

TABLE I. COPPER PRESENT IN APPLE AND CHERRY LEAVES AS DETERMINED BY ELECTROLYTIC METHOD

Type of Leaf	Treatment	Copper Present A	Copper Present B
		Mg./sq. m.	
Apple	1 application of $Cu_3(PO_4)_2$	27.92	27.57
	1 application of Cupro-K	23.08	23.43
	2 applications of copper arsenate	65.31	64.48
Cherry	1 application of $Cu_3(PO_4)_2$	101.55	99.94
	2 applications of basic copper sulfate	26.82	25.21

TABLE II. RECOVERY OF ADDED COPPER SULFATE TO APPLE WASH SOLUTIONS

Copper Added	Copper Recovered	
Mg.	*Mg.*	%
0.050	0.050	100
0.075	0.076	101
0.100	0.098	98
0.150	0.142	95

sults is shown in Table II. Each figure is the average of duplicate determinations. Replicate determinations usually agree within 2 microamperes (about 0.003 mg.).

If a photoelectric colorimeter is not available, the unknown solutions may be compared with standards in a colorimeter. In this case the color should be developed in both standards and unknowns at the same time, since the ability of the colored solution to transmit light decreases slightly on standing.

When using solutions in which no organic matter is present, such as the washings from the plates sprayed in the laboratory, the procedure may be simplified for the sake of rapidity by the elimination of the filtration, if the standardization is carried out under the same conditions.

Summary

Two methods are presented for the rapid determination of copper on surfaces sprayed with insecticide or fungicide mixtures containing this element. The first method, for quantities greater than 2 mg., is a modification of the usual procedure of weighing the metal directly after electrodeposition. The second method, for smaller quantities of copper, is based on the photoelectric measurement of the color produced by sodium diethyl dithiocarbamate.

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THE IMPORTANCE OF STANDARDIZED PROCEDURES IN DILUTING LIQUID LIME SULPHUR[1]

H. W. THURSTON, JR. AND DONALD E. H. FREAR

(Accepted for publication June 20, 1939)

In the use of any fungicide so complex and variable in its composition as liquid lime sulphur, it is difficult at best for the user to be sure of either the fungicidal value or "phytocidal" properties of recommended dilutions. It has been the custom in this country to consider either the specific gravity or Beaumé test of the concentrate as providing a reliable index to the amount of active fungicidal ingredients present. Since, however, the value of lime sulphur probably depends solely on the amount of calcium polysulphides present, while the specific-gravity reading is a function of the total solids in solution, it would seem more logical to base dilutions on the guaranteed polysulphide sulphur content, as is done in England, than upon a specific-gravity or Beaumé reading. Safro,[2] in 1913, was one of the first of many who have pointed out the inadequacy of the Beaumé test as a standard for dilution.

The writers have recently had occasion to analyze 30 samples of lime-sulphur concentrate, representing 8 different commercial brands, and 5 sources of home-boiled concentrate.[3]

These samples are believed to represent a fair cross section of the material available to Pennsylvania fruit growers, and, in all probability, of the country at large.

The analyses are presented in table 1, and reveal several facts of considerable practical importance. It will be noted that there is not a little variation in both the specific gravity and the polysulphide content, the former varying from 1.182 to 1.293 (22.33° to 32.86° Beaumé) and the latter from 10.90 per cent to 25.94 per cent. When these data were analyzed statistically there was found a correlation coefficient of 0.919 between specific gravity and polysulphide content, indicating that specific gravity is, after all, a fairly reliable index of fungicidal value.

Taking as an example the highest (sample 24) and the lowest (sample 2) figures for specific gravity, and assuming that both samples are diluted 1–75, the diluted spray made from sample 2 would contain 0.171 per cent polysulphide sulphur, while a similar dilution of sample 24 would contain 0.445 per cent; more than $2\frac{1}{2}$ times as much. In other words, to get the same final concentration of the active ingredients in the diluted spray, the user would require more than $2\frac{1}{2}$ times as much of the original lime-sulphur concentrate represented by sample 2 as would be used if sample 24 were diluted.

It should be pointed out, on the other hand, that if the procedure of

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[1] Authorized for publication on August 2, 1939 as paper No. 919 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Safro, V. I., An investigation of lime sulphur injury. Oreg. Res. Bull. 2. 1913.

[3] Acknowledgment of their help in collecting the samples, and in carrying through most of the analyses is made to the members of the Extension staff in Plant Pathology at the Pennsylvania State College, and to Mr. James M. Stewart, respectively.

TABLE 1.—*Analysis and source of lime-sulphur samples arranged in order of specific gravity*

Sample number	Specific gravity	Polysulphide sulphur	Thiosulphate sulphur	Source
24	1.293	25.94	1.47	Home-boiled
9	1.283	23.68	2.56	Brand D
23	1.279	24.79	1.55	Brand E
4	1.279	22.73	4.19	Brand B
12	1.278	24.17	1.93	Brand B
28	1.278	23.13	2.09	Brand E
16	1.278	22.92	1.71	Brand E
29	1.272	22.91	1.71	Brand G
27	1.272	22.82	1.78	Brand F
26	1.270	22.95	1.86	Brand E
21	1.270	22.80	2.17	Unknown[a]
7	1.268	21.86	2.17	Brand C
17	1.268	21.78	1.78	Brand F
22	1.267	22.47	2.25	Brand C
18	1.266	21.93	2.02	Brand C
3	1.266	21.57	3.57	Brand A
8	1.265	21.43	2.40	Unknown[a]
14	1.263	22.75	2.17	Brand D
30	1.262	24.47	2.17	Brand E
6	1.262	22.58	2.71	Brand C
13	1.260	23.18	2.33	Brand D
10	1.259	21.48	2.17	Brand D
5	1.258	21.58	4.11	Brand C
20	1.258	21.28	2.09	Brand D
25	1.252	21.81	2.09	Brand D
11	1.250	22.20	2.48	Brand D
19	1.240	17.74	2.95	Home-boiled
1	1.235	15.65	6.05	Home-boiled
15	1.210	13.30	4.50	Home-boiled
2	1.182	10.90	5.27	Home-boiled

[a] Commercial but manufacturer not known.

diluting the concentrate so as to produce a given specific gravity after dilution be used there would be little difference between the two final sprays with regard to their polysulphide sulphur content; it is only when the operator follows the procedure of diluting his lime sulphur year after year to 1–75 or 1–100 or some other dilution, regardless of the specific gravity, that he may find his final spray less effective or more injurious than he hoped.

Thiosulphate Sulphur.—Table 1 indicates that there is a great difference in the thiosulphate-sulphur content of the various samples analyzed. The range of this constituent is between 1.71 per cent and 6.05 per cent, the samples with the lowest specific gravity and polysulphide sulphur content showing in general the highest concentration of thiosulphate sulphur. Statistically, however, there was no correlation between the thiosulphate contents and either the specific gravity or the polysulphide concentrations. Samples 3, 4, 5, and 19 are relatively high in thiosulphate sulphur.

The importance of the thiosulphate-sulphur concentration in lime-sulphur sprays is not too well understood, but there seems to be a general agreement that it is of little or no value as a fungicide, nor does there appear to be any evidence for considering it injurious to foliage.

Commercial vs. Homemade Lime Sulphur.—Five of the 30 samples of lime sulphur collected for this study were homemade; that is, they were boiled by the orchard operator. It is interesting and probably significant that the 4 samples lowest in polysulphides were homemade, and these same 4 samples were among the highest in thiosulphate sulphur. The average polysulphide-sulphur content of the 5 homemade samples was 16.70 per cent, while for the 25 commercial samples it was 22.61 per cent. Thiosulphate sulphur of the homemade samples averaged 4.05 per cent, while the average for the commercial samples was 2.32 per cent.

With one exception, it may be said that the homemade samples were decidedly inferior to the commercial samples. Peculiarly enough, the fifth sample of homemade lime sulphur (sample 24) was the best of the 30 samples examined. This indicates that, with proper care and equipment (in this case a steam heated kettle), lime sulphur of a quality equal or superior to commercial brands may be produced on the farm.

THE PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PENNA.

A scene at the Roseville Tobacco Experimental F

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Mr. Haley is employed in the de-
partment of agricultural and biologi-

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Editor's Note: A second article in this series on fertilizers for Pennsylvania cigar-leaf tobacco will appear in a later issue.

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A scene at the Roseville Tobacco Experimental Field in Pennsylvania this season — a crop grown with 1000 pounds per acre of a 4–8–12 mixture

Planning Fertility for Pennsylvania Cigar-leaf Tobacco

By D. E. Haley and J. J. Reid

Mr. Haley is employed in the department of agricultural and biological chemistry, and Mr. Reid, the division of bacteriology, both at Pennsylvania State College.

IT HAS been evident for a long period of time that both the yield and quality of Pennsylvania cigar-leaf tobacco are controlled in large measure through the rational use of fertilizers. It has been equally evident, however, that the maximum benefits of a given fertilizer treatment are dependent on certain environmental conditions, a few of which will now be mentioned.

The seedbed. It is obvious that extreme care must be taken in the case of seedlings so as to insure a normal and uninterrupted growth and the development of a satisfactory root system. When beds are steamed in the spring in order to kill weed seeds, the heavier types of soil appear to lose their moisture-holding properties. In order to overcome this condition frequent waterings are usually employed. This is unfortunate in that considerable nutrient material is leached from the upper soil layers and the development of the root system is seriously impeded. These conditions result in a disturbance of the mineral balance, which may and sometimes [illegible] further aggravated through the use of stimulants such as manure extracts or dilute solutions of nitrate of soda. Furthermore, the bed may be covered with cloth which will interfere with aeration and help maintain too high a humidity at times. These conditions lead to a highly susceptible condition of the seedlings and aid materially in the transformation of *Pseudomonas fluorescens*, a commonly occurring saprophyte of the soil, to a physiological adaptation known as *Phytomonas tabaci*, or the wildfire organism. The presence of wildfire in a seedbed is a clear indication of the fact that the plants have been grown under undesirable conditions.

Fall steaming of beds appears superior to spring steaming, as normal conditions in respect to moisture-holding properties may be reestablished before seeding. Care should be taken to use fertilizer mixtures similar to those used in the field. The use of sashes is preferable to cloth in that the harmful effects of heavy rains may be controlled. Through raising of the sashes better conditions of aeration are maintained and the danger of high humidities is lessened.

The physical condition of the soil. Attention has been called to the development of a satisfactory root system in the seedbed. Of far greater importance, however, is an unrestricted root development in the field. While our tobacco soils are rather heavy it is possible in most cases to bring them to the proper degree of tilth. Poorly drained soils should never be planted to tobacco.

All growers realize the importance of the physical conditions of their soil, as evidenced in their rotations and liberal use of farm manure. In some cases and under certain conditions the rotation and the use of manure may limit the desirable effects of a given fertilizer treatment.

One of the most critical periods in the life of a tobacco plant is that which immediately follows transplanting. It must establish a good root system and be supplied the necessary mineral elements in the proper quantity and in the proper form. With sufficient rainfall uninterrupted growth may take place and the harmful influence of any rotation or field treatment may be overshadowed. In many cases, however, the field treatment or rotation may definitely check the growth of the young plant.

In order to maintain the proper physical condition of the soil, organic additions are necessary. Formerly, well-rotted mixtures of horse and cow manures were used. Such a practice was fundamentally correct, as this material acted as a reservoir for moisture and mineral nutrients which were available and necessary for the uninterrupted growth of the plant. On the other hand, plowing under old red clover sod or fresh stable manure is not so desirable. These materials, rather than improving the moisture-holding capacity of the soil to the benefit of the plants, compete with them for moisture. On the other hand, weather conditions may be of such a nature that the nutrient materials in these substances may be liberated at a time that the plant is not able to utilize them to advantage, while in the earlier stages of decomposition, the bacteria involved may compete with the plant for nutrient material.

A plant checked in growth may become loaded with starch. Such a condition may be observed during a drought period. If the drought is broken abruptly, nitrogen is readily absorbed, starch disappears and proteins are formed in a series of reactions. Such changes involve a lowering of resistance as evidenced by the appearance of leaf spots. Such a condition would never occur when conditions were satisfactory for normal growth and utilization of the materials absorbed and when uninterrupted growth was in evidence.

In line with the above, an article written by the authors and co-workers entitled "Plant Nutrition and Disease Resistance" may be found in the *Journal of Bacteriology*, Vol. 38, p. 234, and which reads as follows:

"A study has been made of the relation between variations in the nitrogen/mineral uptake of plants and variations in resistance to disease. It has been a common assumption that, other things being equal, the higher the nitrogen level of the plant, the greater the susceptibility of the plant to disease. Data obtained by the authors indicate that this is not the true relation between nitrogen uptake and disease susceptibility.

"The results indicate that it is not a question of how much nitrogen a plant absorbs within reasonable limits that determines susceptibility, but the stage in the life of the plant when significant quantities of nitrogen are absorbed.

"Tobacco plants grown with adequate mineral fertilization may mature with a nitrogen content in excess of four percent and show no significant loss in resistance to leafspot diseases if the nitrogen is supplied without material fluctuations during the stage of active growth. On the other hand it has been found that tobacco plants may show extreme susceptibility to leafspot diseases with a nitrogen content of less than two percent, if the nitrogen is supplied erratically with significant amounts available during the ripening stage.

"It is considered inadvisable, therefore, to employ fresh animal manure, old legume residues and other sources of nitrogen which may provide a fluctuating and late supply of this element in the fertilization of tobacco and other short season crops."

Editor's Note: A second article in this series on fertilizers for Pennsylvania cigar-leaf tobacco will appear in a later issue.

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THE PROTEIN REQUIREMENTS OF THE YOUNG PIG *

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Because of the rapid growth of the pig and its consequent high requirements for specific nutrients, this species shows evidence of specific nutrient deficiencies more quickly and strikingly than either horses, cattle or sheep.

Something of the requirement for specific nutrients of young growing animals is indicated by the composition of the milk of particular species. As given by associates of Rogers (1) the protein content of sows' milk exceeds that of the human, cow, mare, goat and ewe. Hughes (2) has reported the total solids and protein contents of sows' milk to be 17.98 percent and 6.77 percent respectively. Morrison (3) reports these values as 19 percent for total solids and 5.9 percent for protein. From these figures it is indicated that the average feed of a nursing pig contains 31.0 to 37.6 percent of protein on the basis of the dry matter content.

Smith (4) recommends a ration containing 20 percent total protein for pigs between the weights of 30 and 50 pounds. In a study of gains and economy of gains, in which the paired-method was used, Carroll and co-workers (5) reported in favor of pigs fed a 22 percent protein ration as compared to pigs fed a 17 percent protein ration. No evidence of protein poisoning was observed with protein levels as high as 26 percent.

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In a series of three experiments conducted at the Pennsylvania Experiment Station data have been obtained which deal with the protein requirements of pigs from time of weaning until a live weight of 70 pounds is attained.

In Experiments 1 and 2, the pigs were fed in individual stalls in an experimental feed house with concrete floors and concrete outside runways. These pigs were selected in triplicates of the same breed, sex and weight, all members of the same triplicate receiving the same daily quantity of feed. In each trio the quantity of feed allotted to each individual was determined by the pig that consumed the least feed. The pigs were placed in their stalls for feeding three times daily. In these experiments a comparison was made of 12, 17 and 22 percent and 17, 22 and 27 percent protein rations.

In the third experiment a comparison was made of 9, 12, 15 and 18 percent protein rations for pigs grazing on Dwarf Essex rape forage. These pigs were selected in quadruplicates of the same breed, sex and weight, and were allotted feed and fed their grain in individual stalls in the same manner as the triplicates were fed. The pigs grazed on the rape forage continuously except the three or four hours of each day spent in the stalls.

The composition of the rations is shown in table 1. Alfalfa constituted 4 percent of the ration of Experiment 1, 10 percent of the ration of Experiment 2, but was not included in Experiment 3, with rape forage.

TABLE 1

RATIONS AND CONSTITUENTS

Feeds	Experiments									
	1			2			3			
	Protein level			Protein level			Protein level			
	12	17	22	17	22	27	9	12	15	18
	Percent			Percent			Percent			
Yellow corn	87.5	69.5	61.5	65.5	45.5	36.5	99.0	87.0	76.0	68.0
Tankage 34%	4.0	13.0	22.0	12.0	22.0	20.0		9.0	15.0	11.0
Soybean oilmeal	4.0	13.0	22.0	12.0	22.0	33.0		3.0	8.0	20.0
Alfalfa meal	4.0	4.0	4.0	10.0	10.0	10.0				
Sodium chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Limestone							0.5	0.5	0.5	0.5

In summarizing the data on the gains of pigs between the weights of 40 and 70 pounds, in Experiments 1 and 2, the final weights of the pigs were taken as the first weekly weight after the pigs had reached a weight of 70 pounds and in Experiment 3 as the weekly weight closest to 70 pounds.

Table 2 presents the initial weights, the final weights, the average daily gains, the time required to reach 70 pounds in live weight, the average daily rations and the economy of gain.

TABLE 2

AVERAGE WEIGHTS, GAINS AND FEED REQUIREMENTS

Number of pigs	Protein percent.	Period	Initial weight	Final weight	Aver. daily gain	Aver. daily ration	Feed per 100 lbs. gain
		days	pounds	pounds	pounds	pounds	pounds
Experiment 1—Fed on concrete							
9	12	76	40	74	.45	1.56	375
12	17	72	38	75	.50	1.43	301
12	22	70	41	74	.51	1.31	274
Experiment 2—Fed on concrete							
10	17	54	33	74	.75	1.85	271
10	22	51	32	70	.74	1.94	270
10	27	49	34	74	.78	1.98	254
Experiment 3—Fed on rape forage							
9	9	52	36	72	.72	1.74	255
9	12	52	35	73	.73	1.76	247
9	15	44	36	68	.74	1.54	217
9	18	42	37	72	.84	1.58	188

The 12 percent protein ration of Experiment 1 produced the smallest and least economical gains, with more time and more feed being required to produce 10 percent less gain on this ration as compared to the 17 and 22 percent protein rations. Of the 13 triplicates, in six cases the pigs fed the 22 percent protein ration gained more rapidly and required less feed per unit of gain; in six triplicates the pigs fed the 17 percent protein ration had the highest and the most economical gains. Four pigs fed the 12 percent protein ration did not reach 70 pounds in weight, as compared to one pig fed the 17 percent protein ration and one pig fed the 22 percent protein ration. The data on the performance of these pigs were not used. The pigs fed the 22 percent protein ration made .01 pound greater average daily gain on nine percent less feed per unit of gain than the pigs fed the 17 percent protein ration.

The pigs fed the 27 percent protein ration, in Experiment 2, made the highest average daily gain in the least time on approximately six percent less feed per unit of gain. All members of all triplicates reached the weight of 70 pounds in live weight within 63 days or less. Of the 10 triplicates, in three cases the pig fed the 27 percent protein ration made the most economical gains; in four cases the pig fed the 22 percent protein ration made the most economical gains; and in three cases the pig fed the 17 percent ration made the most economical gains.

In Experiment 3, the pigs fed the 18 percent protein ration on Dwarf Essex rape gained 14, 13 and 12 percent more per day and required 36, 31 and 15 percent less feed than the pigs fed the 9, 12 and 15 percent protein rations respectively. The pigs fed the 18 percent protein ration required 10 days less time to reach a weight of 72 pounds than the pigs fed the 12 and 9 percent protein ration.

In Experiments 1 and 2 the efficiency of feed utilization as shown by the quantity of feed required for 100 pounds of body gain is very high, ranging from 254 to 375 pounds. Woodman and others (6) have shown that with pigs fed individually as compared to group fed pigs, the former had a higher efficiency of food utilization and were more thrifty than the group fed pigs. These differences were ascribed by these workers to greater expenditure of energy by the group fed pigs in struggling for feed at meal time and to closer control of feeding enabled by the individual feeding procedure.

The figures on feed requirements reported in these experiments are very much lower than usually reported in this country and are lower than those reported by Woodman for individually fed pigs.

The idea of conservation of energy in the feeding procedure as proposed by Woodman may partially explain this situation, but in addition it may be pointed out that two out of three pigs of each trio were limited in feed intake even though such limitation may have been small in some cases, and that under such conditions of limited feed intake a higher efficiency of food utilization may result because of the use of nutrients for tissue building and not for the mere satisfaction of appetite.

While the conditions of limited feed intake may not fully reflect the complete nutritive effect of a ration in all its aspects, the conditions imposed in these experiments gave a very close approach to the true comparative value of the rations being studied in this project.

In Experiment 1, the average daily feed consumption was approximately 2.5 pounds per day for each 100 pounds of live weight on the average, while in Experiment 2, the daily feed consumption might be considered approximately normal, being 3.2 pounds per day for each 100 pounds of live weight. In both experiments these figures do not represent the maximum food intakes possible on these rations, but rather are limited to a degree imposed by this method of feeding.

Summary

A study has been made of the growth response and efficiency of food utilization of the young pig from the time of weaning to a live weight of 70 pounds, fed on rations containing from 9 to 27 percent protein, low protein abattoir tankage being used in conjunction with soybean oil meal and alfalfa meal as a supplement to yellow corn.

The growth response was not satisfactory on the 12 percent protein rations without forage. Satisfactory growth was obtained on the 17, 22, and 27 percent protein rations of the pigs fed on concrete, and on 9, 12, 15, and 18 percent protein rations of the pigs fed the grain rations on Dwarf Essex rape pasture.

Maximum gains and most economical gains were made by those pigs fed the rations with the highest percentages of protein.

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